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RESEARCH INVESTIGATIONS

ANAPHYLACTOID PHENOMENA FROM THE INTRAVENOUS ADMINISTRATION OF VARIOUS COLLOIDS, ARSENICALS AND OTHER AGENTS *

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AND

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CLEVELAND

(From the Journal of Pharmacology and Experimental Therapeutics, January, 1920, p. 379)

I. INTRODUCTION

The object of this study is to throw additional light on the dangers of intravenous medication and the relationship of these to anaphylaxis and anaphylactic shock by a detailed study of the effects of the intravenous administration of a variety of agents, principally colloids of non-protein origin. Before proceeding to the results of our experiments, a brief consideration of the fundamental aspects of the problem is desirable.

The intravenous injection of certain agents such as serum, diphtheria antitoxin, foreign proteins and arsphenamin produce in some individuals disturbances which resemble the symptoms of anaphylactic shock. It is alleged that in these individuals a state of anaphylaxis exists or, at least, an unusual degree of sensitiveness, presumably the state of sensitization, although in many instances such individuals have not been previously treated with the agent. This is particularly true of such agents as arsphenamin, while with

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proteins, no matter what the source, sensitization might conceivably exist.

This is rendered less probable in view of the fact that similar responses, which may be conveniently designated as "anaphylactoid phenomena," occur after the injection of various non-protein colloids into previously untreated or normal animals. Among such agents are kaolin, starch and inulin, and agar, previously investigated by Friedberger,[1] Nathan [2] and Bordet,[3] and Novy and DeKruif,[4] respectively. For instance, agar, a non-protein colloid and otherwise physiologically nontoxic and inert, when injected intravenously into a guinea-pig in the form of a sol-fel (1 : 6) in the very small dosage of 0.0094 mgm. agar per gram of animal produces symptoms indistinguishable from true anaphylactic shock and death and at autopsy the lungs are markedly distended. In fact, Novy, DeKruif and Novy [5] state that agar in this way is 33 times as toxic as peptone for the guinea-pig. From this fact, and, also, since proteins are colloids, it has been alleged [6] that the anaphylactoid symptoms, if not anaphylactic shock itself, are essentially a colloidal phenomenon. The explosiveness of the symptoms is said to be brought about by some disturbances in the aggregates of the colloids themselves, of the blood colloids and even in the cells free from blood and lymph. A review of the extensive work that has been done on anaphylaxis renders it evident that by leaning on this view exclusively it is difficult to reconcile the effects produced by certain substances with the state of true anaphylaxis and the phenomena of anaphylactic shock merely by the close resemblance of the symptoms. Solutions of arsphenamin have been brought into the category of colloids by Danysz,[7] who claims that the base is precipitated out by constituents of the blood, and again formed into soluble organic derivatives in the circulation. The disturbances accompanying arsphenamin injections are said to be associated with the rapidity of this precipitation, the rate depending on the alkalinity and peculiarities of individual plasma.

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[1] Friedberger and Tsuneoka: Ztschr. f. Immunitäts f. u. exp. Therap. **20:** 405.

[2] Nathan: Ztschr. f. Immunitätsforsch. **23:** 204, 1914.

[3] Bordet: Compt. rend. soc. de biol. **75:** 225, 1913.

[4] Novy and DeKruif: J. Inf. Dis. **20:** 536, 117.

[5] Novy, F. G.; DeKruif, P. H., and Novy, F. O.: J. Inf. Dis. **20:** 657, 1917.

[6] Kitchewsky: J. Inf. Dis. **22:** 101, 1918. Russkiy Vratch, **15**, No. 4, 1916, Bronfenbrenner: Proc. Soc. Exp. Biol. Med. **13:** 19, 1915.

[7] Danysz: Ann. de l'Inst. Pasteur, **21:** 114, 1917.

It was, therefore, resolved to investigate a variety of agents to see if these could be classed according to whether they possessed colloid characteristics or not. A number were found to act outwardly similar to agar. Finally, it was deemed necessary to investigate in particular the symptoms of lung inflation from agar and similar agents, for, if this could be substantiated with surviving lungs and if stimulation of smooth muscle in other regions could be demonstrated, namely, in the intestines and uterus, then indeed it would appear as if the explanation of "shock" on a colloidal basis had some foundation in fact. This might also explain the accidents and disturbances accompanying the intravenous administration of sundry agents, including arsphenamin.

With few exceptions, the tendency of the investigations concerning agents producing anaphylactoid phenomena appears to be limited almost exclusively to the symptomatology. Owing to its close relation to all physiological functions, and the respiratory function in particular, it is obvious that the circulation should not be left out of consideration. If precipitations in the blood stream occur, as alleged from in vitro experiments, these should be looked for microscopically and otherwise.

We have attempted to cover all these features. That is, the symptoms together with the changes at autopsy and microscopic examinations of the lungs, agglutination, and responses of surviving bronchial, intestine and uterine musculature have been taken into consideration. In this paper, the results of intravenous injections in guinea-pigs are recorded and the various agents classified. The remaining features will be presented in subsequent papers.

II. METHODS

Guinea-pigs of the average of 300 grams body weight were used throughout. A small skin incision was made, using a bare whiff of ether as anesthesia, for exposure of the jugular veins. The majority of injections were made into the left jugular veins, sometimes into the right vein. The injections were made slowly, consuming about 60 seconds for 3 cc. of solution. All agents were suspended or dissolved in 0.9 per cent. NaCl and injected at about 38 C. Immediately after the injection, a small bulldog clip was placed on the vein, the animal was untied and observed. The volume of solution injected was 3 cc. as a rule, but this was varied from time to time to suit special purposes. Assuming that a 300 gm. guinea-pig contains about 6 per cent. of blood, or 18 gm., the volume injected would constitute about 18 per cent. additional fluid which is not regarded as unduly diluting the blood or burdening the circulation.

As a rule the concentrations and doses of the different agents used represented those used in therapeutic practice, except in the case of non-therapeutic agents with which enough was used to produce effects. Before injection each solution was filtered through a quantitative filter (Whatman No. 40). The pollen extracts, phylacogen, typhobacterin and venarsen were used as such from the original containers. The solutions of arsphenamin and neoarsphenamin were prepared according to the directions accompanying each preparation.

Agar sol, agar sol-gel and toxified agar were prepared according to the directions of Novy [4] and collaborators. Agar sol is a 0.5 per cent. suspension of agar in saline. This solidifies on cooling and must be melted before injection. When 1 part of agar sol is melted and diluted to 4 parts or 6 parts with normal saline, and is cooled in cracked ice for two hours, then thoroughly shaken, this constitutes agar sol-gel 1 : 4 and 1 : 6, respectively. Toxified agar was prepared by heating 1 part of agar sol (0.5 per cent.) on a water bath for two hours and adding to it 1 part of serum (rabbit's in our work) and incubating at 38 C. for fifteen minutes. The preparation is then ready for injection. Toxified acacia was prepared in the same way.

The acacia used in our work was the Turkey or Khardofan variety in small tears. The solutions were prepared by boiling for half an hour and then filtered in the usual way.

Practically all animals that survived the injections, or did not die shortly thereafter, were killed at the end of half an hour, some somewhat later, a few sooner. Autopsy was then performed, carefully examining the presence or absence of pulmonary distention, hemorrhages and congestion; the condition of the heart; coagulation of blood and absence or presence of visceral congestion.

Suitable sections of lungs were made and these were placed at once, while still fresh, into Zenker's fluid, for microscopic examination as to the absence or presence of bronchial distention, thrombi, conglutination and hemorrhages.

In the majority of experiments, 3 animals were injected with the different agents. All told thirty-one different agents were studied, two of which, acacia and agar, were injected in three and four different forms, respectively. In all seventy-one animals (seventy guinea-pigs and one rabbit) were injected.

III. RESULTS AND DISCUSSIONS

The results with various thromboplastic agents, namely, the thromboplastins, coagulen and hemostatic serum, have been described in a previous paper.[8] The detailed results of the present series are presented in Table 1 and summarized and classified as to principal phenomena produced in Table 2. These may now be described.

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[8] Hanzlik, Karsner and Fetterman: J. Pharm. Exp. Therap., **14**: 229, 1919.

TABLE 1.—EFFECTS PRODUCED BY THE INTRAVENOUS INJECTION OF VARIOUS COLLOIDS AND OTHER AGENTS IN GUINEA-PIGS

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm.	Principal Symptoms	Fate of Animal (End of Minutes)	Necropsy	Microscopic Examination of Lungs
				Saline Controls (0.9 per cent. NaCl)			
1	...	2	....	None.........................	Killed, 20	Lungs collapsed...............	Normal
7	350	10	....	None.........................	Killed, 86	Lungs collapsed...............	Normal
46	235	3	....	None.........................	Killed, 48	Lungs partially inflated.......	Marked congestion
				(0.85 per cent. C. P. NaCl)			
52	220	3	....	None.........................	Killed, 41	Lungs collapsed; pulmonary congestion; slight cardiac dilatation	Marked congestion; thrombosis of few large veins
				Agar sol 0.5 per cent.			
2	200	2	0.05 mg.	Dyspnea; depression; trembling; death	Fatal, 12	Inflated lungs	
4	215	2	0.046 mg.	Respiration stopped; relaxation of sphincters; death	Fatal, 5	Lungs inflated and hemorrhagic	Congestion, hemorrhage, thrombosis, edema
				Agar sol gel (1 :6)			
88	320	5	0.013 mg.	Marked dyspnea and depression; convulsions; increased respiration; death	Killed, 31	Marked pulmonary distention and hemorrhages and slight congestion; moderate cardiac dilatation; moderate abdominal congestion Blood clots rapidly	Marked distention, marked congestion, marked hemorrhage, thrombosis
				Agar sol gel (1 : 4)			
19	200	3	0.015 mg.	Marked dyspnea; spasms cyanosis; death	Fatal, 21	Marked pulmonary inflation; pale; no cardiac dilatation; no abdominal congestion	Marked distention, slight congestion, thrombosis
				Agar gel (0.5 per cent. agar 1 part and N. S. 1 part)			
13	200	2	0.025 mg.	Dyspnea; jerky spasms......	Killed, 35	Slight pulmonary inflation; hemorrhages; heart normal	Moderate distention, congestion, hemorrhage, edema, thrombosis
				Toxified agar (1 part of 0.5 per cent. agar and 4 parts of rabbit serum)			
3	200	2	0.01 mg.	Dyspnea; air hunger; depression	Killed, 3 hrs., 9 min.	Marked pulmonary inflation	
6	205	2	0.01 mg.	Increased respiration spasms, dyspnea moderate	Killed, 3 hours	Lungs inflated.................	Congestion, hemorrhage, thrombosis

Acacia (6 per cent. and 0.5 per cent.)							
5	200	2 c.c. of 0.5 per cent.	0.05 mg.	Dyspnea; spasms; convulsions	Killed, 4 hrs., 12 min.	Lungs partially inflated.......	Congestion, conglutination thrombi
53	205	3 c.c. of 6 per cent.	0.88 mg.	Dyspnea; restlessness respiratory rate increased	Killed, 31	Lungs collapsed; no hemorrhages; no cardiac dilatation	Marked congestion, capillary conglutination thrombi
9	168	4 c.c. of 0.5 per cent.	0.12 mg.	Dyspnea; trembling; depression	Killed, 38	Slight pulmonary inflation; hemorrhage; cardiac dilatation; liver dark	Congestion, conglutination thrombi
14	260	3 c.c. of 6 per cent.	0.69 mg.	Dyspnea; spasms; increased respiration	Killed, 35	Lungs inflated (partial); no cardiac dilatation	Marked distention, congestion, hemorrhage, edema, slight thrombosis
29	180	3 c.c. of 6 per cent.	1 mg.	Marked dyspnea.............	Killed, 31	Lungs collapsed; fine punctate hemorrhages; slight cardiac dilatation; no abdominal congestion	Moderate distention, congestion, thrombosis
81	380	2.8 c.c. of 6 per cent.	0.44 mg.	Increased respiration; restlessness; moderate dyspnea	Killed, 33	Marked pulmonary distention, hemorrhages and moderate congestion; moderate cardiac dilatation	Marked distention, marked congestion, marked hemorrhage
Toxified acacia (1 part of acacia 6 per cent. and 2 parts of rabbit serum)							
27	175	3	0.34 mg.	Marked dyspnea; respiration slowed, restlessness, depression	Killed, 32	Marked pulmonary inflation; hemorrhages; cardiac dilatation, no abdominal congestion	Marked distention, congestion, hemorrhage, thrombosis
Dialyzed acacia (6 per cent.)							
45	360	3	0.5 mg.	Dyspnea; respiration slowed, discomfort	Killed, 30	Marked pulmonary distention, congestion and minute hemorrhages; cardiac dilatation	Marked congestion, slight hemorrhage
Beef serum (whole; fresh)							
10	145	2	0.014 c.c.	Respiration and heart stopped; death	Fatal, 2	Marked pulmonary inflation; hemorrhages marked; marked cardiac dilatation	Marked distention, congestion, hemorrhage, conglutination thrombi
Dog bile (whole; fresh)							
11	203	2	0.01 c.c.	Marked depression; respiration slowed; death	Fatal, 6	Marked pulmonary inflation; hemorrhages; heart normal	Moderate distention, congestion, bile emboli and thrombosis
Gelatin (0.5 per cent. and 6 per cent.)							
153	500	3 c.c. of 6 per cent.	0.35 mg.	None........................	Killed, 35	Lungs collapsed and congested; cardiac dilatation	Marked congestion, capillary conglutination thrombi, only slight local distention

TABLE 1. EFFECTS PRODUCED BY THE INTRAVENOUS INJECTION OF VARIOUS COLLOIDS AND OTHER AGENTS IN GUINEA-PIGS.—Continued

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm.	Principal Symptoms	Fate of Animal (End of Minutes)	Necropsy	Microscopic Examination of Lungs
Gelatin (0.5 per cent. and 6 per cent.)—Continued							
15	200	3 c.c. of 0.5 per cent.	0.075 mg.	Slight dyspnea; jerky.......	Killed, 35	Partial or no pulmonary inflation; no cardiac dilatation, no abdominal congestion	Moderate distention, marked congestion, marked hemorrhage
44	260	3 c.c. of 6 per cent.	0.7 mg.	Depression; dyspnea; respiration increased	Killed, 31	Slight pulmonary distention; marked congestion, some hemorrhages; marked cardiac dilatation	Congestion, hemorrhage
Starch (2 per cent. and 6 per cent.)							
154	450	3	0.4 mg.	Dyspnea; increased respiration	Killed, 47	Lungs partially distended, markedly congested; moderate cardiac dilatation	Moderate general distention, slight congestion, marked hemorrhage
16	205	3 c.c. of 2 per cent.	0.29 mg.	None........................	Killed, 32	Partial or no pulmonary inflation	Marked congestion, marked hemorrhage
						Hemorrhage upper right lobe; cardiac dilatation	Marked congestion, marked hemorrhage
46a	330	3 c.c. of 6 per cent.	0.54 mg.	Convulsions; marked dyspnea	Killed, 30	Marked pulmonary inflation, hemorrhages and congestion; cardiac dilatation	
Collargol (1 per cent. and 5 per cent.)							
17	205	3 c.c. of 5 per cent.	0.73 mg.	Respiration stopped; death	Fatal, 4	Slight pulmonary distention; blood granular; cardiac dilatation; abdominal congestion; blood granular	Marked congestion, marked thrombosis
20	175	2.8 c.c. of 1 per cent	0.16 mg.	Marked depression; respiration slowed; cyanosis, pupils dilated, facial twitching, death	Fatal, 30	Slight pulmonary distention; hemorrhage, blood less granular; cardiac dilatation; clot in heart	Moderate congestion, conglutination, thrombi
36	245	0.1 c.c. of 2 per cent.	0.0081 mg.	Depression; dyspnea marked	Killed, 30	Lungs collapsed and congested; no abdominal congestion, heart slightly dilated	Marked distention, slight congestion, hemorrhage

				Peptone (Witte; 5 per cent. and 10 per cent.)			
28	160	3 c.c. of 5 per cent.	0.94 mg.	Dyspnea; convulsions; cyanosis; pupils dilated, relaxation of sphincters; death	Fatal, 7	Marked pulmonary inflation; lungs pale; no hemorrhages; marked cardiac dilatation; slight abdominal congestion	Marked distention
89	530	0.1 c.c. of 10 per cent. diluted to 3 c.c. of N. S.	0.019 mg.	Some dyspnea; increased respiration	Killed, 42	Marked pulmonary distention, congestion and hemorrhages; cardiac dilatation; no abdominal congestion	Marked distention, marked congestion, marked hemorrhage
				Dextrin (6 per cent. and 1 per cent.)			
155	460	3	0.4 mg.	Moderate dyspnea..........	Killed, 35	Lungs collapsed, congested and hemorrhagic; moderate cardiac dilatation; abdominal congestion	Marked congestion, no distention
25	185	3 c.c. of 6 per cent.	About 1 mg.	Depression; dyspnea respiration slowed	Killed, 40	Pulmonary inflation doubtful; marked hemorrhages	Marked distention, marked congestion, marked hemorrhage, edema
87	410	3 c.c. of 1 per cent.	0.073	Dyspnea (?) restlessness.....	Killed, 33	Slight pulmonary distention, congestion and numerous hemorrhages marked cardiac dilatation; moderate abdominal congestion	Marked distention, marked congestion, marked hemorrhage
				Pancreatin (1 per cent.)			
Rabbit	1 kg.	5	0.053 gm. × kg.	Shivering; spasms; increased respiration; dyspnea; depression	Killed, 2 hrs.	Distention of lungs; hemorrhages marked; abdominal congestion	
37	300	1.5 c.c. of 1 per cent. diluted to 3 c.c. N. S.	0.05 mg.	Marked dyspnea; restlessness; increased respiration	Killed, 37	Lungs inflated and hemorrhagic; no cardiac dilatation; no abdominal congestion	Moderate distention, marked congestion
				Typhobacterin (Mulford)			
32	200	3	0.015 c.c.	Jerky spasms; restlessness; dyspnea; ruffling of hair	Killed, 30	Marked inflation of both lungs; hemorrhage left lung, also congestion and pneumonia right lung; no abdominal congestion	Moderate congestion
				Pollen extracts (Mulford)			
31	245	2.7 c.c. (ragweed)	0.011 c.c.	Depressed; dyspnea marked	Killed, 30	Lungs collapsed and somewhat congested; heart somewhat dilated; no abdominal congestion	Marked congestion, slight hemorrhage

TABLE 1.—EFFECTS PRODUCED BY THE INTRAVENOUS INJECTION OF VARIOUS COLLOIDS AND OTHER AGENTS IN GUINEA-PIGS.—Continued

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm.	Principal Symptoms	Fate of Animal (End of Minutes)	Necropsy	Microscopic Examination of Lungs
				Pollen extracts (Mulford)—Continued			
47	210	2.7 c.c. (ragweed)	0.012 c.c.	Marked depression; dyspnea; cyanosis; respiration increased	Killed, 33	Partial distention and congestion of lungs; left cardiac dilatation; no abdominal congestion	Marked congestion, slight hemorrhage
84	310	1 (hay fever spring; mixed pollens)	0.000064 mg. Pollen-nitrogen	Moderate dyspnea..........	Killed, 42	Marked pulmonary distention, hemorrhages and slight congestion; slight cardiac dilatation; moderate abdominal congestion	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi
85	530	1 (hay fever fall; mixed pollens)	0.000037 mg. Pollen-nitrogen	Increased respiration; dyspnea; restlessness	Killed, 40	Lungs collapsed; marked pulmonary congestion; no hemorrhage; marked cardiac dilatation; slight abdominal congestion	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi
96	440	1 (hay fever fall; mixed pollens)	0.000022 mg. Pollen-nitrogen	Dyspnea; marked increase in respiration	Killed, 48	Pulmonary distention, marked congestion and multiple hemorrhages; marked cardiac dilatation; moderate abdominal congestion	Marked congestion
				Arsphenamin (0.5 per cent.)			
35	200	0.2 c.c. in 3 c.c. N. S. (D. R. L.)*	0.0075 mg.	Marked dyspnea; respiration slowed; depression; hair ruffled	Killed, 32	Lungs collapsed and markedly congested; cardiac dilatation both sides, no abdominal congestion	Marked congestion, slight hemorrhage
34	235	0.33 c.c. (D. R. L.)	0.007 mg.	Dyspnea; hair ruffled; jerky; shivering	Killed, 42	Lungs collapsed and congested; cardiac dilatation; no abdominal congestion	Marked distention, marked congestion, marked hemorrhage
105	400	0.7 c.c. (T. L.)†	0.009 mg.	Marked dyspnea; choking; restlessness	Killed, 41	Lungs collapsed and congested with few small hemorrhages, slight cardiac dilatation and moderate abdominal congestion; blood clots in 2 minutes	Marked distention, marked congestion, marked hemorrhage, conglutination thrombi

				Glycogen (0.5 per cent.)			
38	350	3.5	0.05 mg.	Depression; dyspnea; spasms; restlessness	Killed, 37	Marked pulmonary distention and congestion; pneumonia right lung; cardiac dilatation; no abdominal congestion	Slight distention, moderate congestion
86	240	3	0.065 mg.	Dyspnea; sick..............	Killed, 30	Pulmonary distention and congestion; marked cardiac dilatation and abdominal congestion	Slight distention, marked congestion
				Nuclein solution (Abbott; about 0.02 per cent.)			
40	200	1.4 c.c. diluted to 3 c.c. N. S. (old)	0.0014 mg.	Depression; respiration slow and shallow; no dyspnea	Killed, 34	Very slight distention of lungs; considerable congestion; no cardiac dilatation; no abdominal congestion	Marked congestion
104	330	0.5 c.c. (fresh)	0.0015 c.c. or 0.00033 mg.	Marked increase in respiration; restlessness; no dyspnea	Killed, 45	Slight pulmonary congestion, marked hemorrhages and moderate congestion; moderate cardiac dilatation; and abdominal congestion	Moderate congestion, slight hemorrhage
115	430	3 (fresh)	0.009 c.c. or 0.0032 mg.	Increased respiration; no dyspnea	Killed, 31	Lungs markedly distended, congested and hemorrhagic; moderate cardiac dilatation and abdominal congestion; blood clotted in 2 minutes	Marked distention, marked congestion, marked hemorrhage
				5 per cent. Inulin (1:10 N. S.; suspension)			
39	210	2.1 c.c. diluted to 3 c.c.	0.05 mg.	Marked dyspnea; respiration shallow; cyanosis; restless	Killed, 30	Lungs collapsed and congested; cardiac dilatation; no abdominal congestion	Marked congestion, slight hemorrhage
83	370	3	0.81 mg.	Marked dyspnea; increased respiration; restlessness	Killed, 43	Lungs collapsed and congested; no hemorrhages; marked cardiac dilatation	Moderate congestion
				Neoarsphenamin (0.6 per cent.)			
43	220	0.55 c.c. to 3 c.c. N. S. (S. D. C.)‡	0.015 mg.	Increased respiration; marked dyspnea; spasms	Killed, 31	Lungs collapsed; marked congestion; dilatation right heart; no abdominal congestion	Moderate congestion, hemorrhage
33	260	0.65	0.015 mg.	Dyspnea; increased respiration	Killed, 38	Pulmonary distention, congestion and hemorrhages; slight cardiac dilatation; slight abdominal congestion	Moderate distention, marked congestion, marked hemorrhage

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* D. R. L. = Dermatological Research Laboratories.

† T. L. = Takamine Laboratory Inc.

‡ S. D. C. = Synthetic Drug Co.

TABLE 1.—EFFECTS PRODUCED BY THE INTRAVENOUS INJECTION OF VARIOUS COLLOIDS AND OTHER AGENTS IN GUINEA PIGS.—Continued

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm.	Principal Symptoms	Fate of Animal (End of Minutes)	Necropsy	Microscopic Examination of Lungs
				Neoarsphenamin (0.6 per cent.)—Continued			
108	440	1.1 c.c. (T. L.)†	0.015 mg.	None.........................	Killed, 30	Lungs slightly distended, congested and hemorrhagic; moderate cardiac dilatation and abdominal congestion	Marked congestion
				Sodium arsenate (0.6 per cent.)			
49	285	0.40 c.c. to 3 c.c. N. S.	0.0084 mg.	Depression; respiration slowed; dyspnea; cyanosis; restless	Killed, 32	Slight pulmonary distention and congestion; slight cardiac dilatation, slight abdominal congestion	Moderate distention, moderate congestion, hemorrhage
103	350	0.22 c.c. to 3 c.c. N. S.	0.0038 mg.	Increased respiration; hair ruffled	Killed, 33	Lungs collapsed and congested; marked cardiac dilatation; very slight abdominal congestion	Moderate distention, marked congestion, marked hemorrhage, thrombosis, edema
				Rabbit's serum (dialyzed)			
47	330	3	0.0091 mg.	Dyspnea; restlessness; respiration increased	Killed, 48	Slight pulmonary distention and marked congestion; heart dilated; slight abdominal congestion	Marked congestion, slight hemorrhage
				Ether and operation (control)			
58	280	No intravenous injection	....	None.........................	Killed, 37	Lungs collapsed; distinct congestion; no cardiac dilatation; slight abdominal congestion	Marked congestion, slight hemorrhage
				Human serum (pooled)			
62	190	3	0.016 c.c.	Dyspnea; convulsions; cyanosis; depression	Killed, 34	Marked pulmonary distention; hemorrhages and edema in lungs; slight cardiac dilatation; no abdominal congestion	Moderate distention, marked congestion, thrombosis
				Congo red (1 per cent.)			
80	400	3	0.075 mg.	Increased respiration; dyspnea	Killed, 44	Marked pulmonary distention, hemorrhages and congestion; marked cardiac dilatation; moderate abdominal congestion	Marked distention marked congestion, hemorrhage

90	220	1	0.045 mg.	Increased respiration; slight dyspnea	Killed, 40	Slight pulmonary distention, considerable congestion, no hemorrhages, cardiac dilatation; abdominal congestion	Marked congestion
151	460	3	0.065 mg.	Marked depression; dyspnea twitching; death	Fatal, 23	Lungs collapsed and moderately congested; marked cardiac dilatation and abdominal congestion	Marked congestion, slight local distention
Althea (15 per cent. extract)							
82	300	3	1.5 mg.	Some increase in respiration	Killed, 35	Very slight distention of lung; marked congestion and slight cardiac dilatation; marked abdominal congestion	Marked congestion. conglutination thrombi
109	440	3	1.02 mg.	Increased respiration; dyspnea	Killed, 36	Lungs markedly distended congested and hemorrhagic; marked cardiac dilatation and abdominal congestion; blood clots in 3 minutes	Marked congestion
152	420	3	1.07 mg.	Marked increase in respiration; twitching of ears	Killed, 33	Lungs congested markedly and collapsed; cardiac dilatation; moderate abdominal congestion	Moderate congestion, few platelet thrombi, no distention
Venarsen (sodium cacodylate)							
95	220	0.07 diluted to 0.5 c.c. with saline (I. Co.)*	....	None........................	Killed, 44	Lungs collapsed and slightly congested, very sight abdomial congestion	Marked congestion, slight hemorrhage
Phylacogen (mixed infection; Parke Davis Company)							
94	260	1	0.0035 c.c.	Restlessness; shivering; increased respiration	Killed, 36	Slight pulmonary distention; marked congestion and minute hemorrhages; slight cardiac dilatation and abdominal congestion	Slight distention, marked congestion, slight hemorrhage, slight conglutination thrombosis
102	500	1	0.002 c.c.	Increased respiration........	Killed, 37	Lungs collapsed; few minute hemorrhages, considerable congestion, moderate cardiac dilatation and abdominal congestion	Marked congestion, slight hemorrhage
110	380	2	0.0053 c.c.	Very marked dyspnea; increased respiration; restlessness; shivering	Killed, 34	Very little pulmonary distention, slight congestion and hemorrhages; slight cardiac dilatation; blood clots in 2 minutes	Marked distention, marked congestion, marked hemorrhage

* I. Co. = Intravenous Products Co.

T. L. = Takamine Laboratory Inc.

TABLE 2.—CLASSIFICATION OF AGENTS AS TO INJURY TO THE CIRCULATION AND RESPIRATION IN THE MAJORITY OF ANIMALS

Injury	Criteria	Agents and Remarks
Practically harmless	Anaphylactoid symptoms absent Pulmonary distention absent macroscopically and microscopically Pulmonary congestion present in some, hemorrhage absent in all Pulmonary thrombi absent Cardiac dilatation absent or unimportant	Ether anesthesia and operation alone (pulmonary congestion present) Normal saline (0.9 per cent. NaCl)
Respiratory system chiefly	Anaphylactoid symptoms present Pulmonary distention present macroscopically and microscopically Pulmonary congestion and hemorrhage present Pulmonary thrombi generally present Cardiac dilatation absent or doubtful	Agar sol (fatal) Ager gel Agar sol-gel (fatal) Agar toxified with rabbit's serum Bile (dog's; fatal) Human serum (thrombi absent) Typhobacterin (thrombi and congestion absent)
Circulatory system chiefly	Anaphylactoid symptoms usually present Pulmonary distention absent macroscopically and microscopically Pulmonary congestion and hemorrhage present Pulmonary thrombi generally absent Cardiac dilatation present	Althea extract (thrombi in 2 out of 3 animals) Arsphenamin (microscopic distention; thrombi in 1 out of 3 animals) Collargol (fatal; microscopic distention and thrombi present in 2 out of 3 animals) Congo red (fatal; microscopic distention in 1 out of 3 animals) Dextrin (microscopic distention) Gelatin (symptoms absent in 1 animal; conglutination present in 1 animal) Inulin
Circulatory system chiefly	Anaphylactoid symptoms usually present Pulmonary distention absent macroscopically and microscopically Pulmonary congestion and hemorrhage present Pulmonary thrombi generally present Cardiac dilatation present	Neoarsphenamin (microscopic distention in 1 out of 3 animals) Phylacogen (microscopic distention in 2 out of 3 and thrombi in 1 out of 3 animals) Rabbit serum Sodium arsenate (symptoms absent in 1 and thrombi present in 1 out of 2 animals) Venarsen (symptoms absent)
Circulatory and respiratory systems	Anaphylactoid symptoms present Pulmonary distention present macroscopically and usually microscopically Pulmonary congestion and hemorrhage generally present Pulmonary thrombi present or absent Cardiac dilatation present, usually also abdominal congestion	Acacia 50 per cent., 6 per cent. or less Acacia dialyzed (no microscopic distention) Acacia toxified with rabbit's serum (no microscopic distention) Beef serum (fatal; thrombi present) Glycogen (microscopic distention and thrombi absent) Nuclein solution (no thrombi) Pancreatin (thrombi absent) Peptone (Witte; fatal; thrombi absent) Pollen extracts (thrombi and microscopic distention in 2 out of 5 animals) Starch (microscopic distention in 1 out of 3 animals and thrombi absent)

1. *Practically Harmless Agents*

Out of the thirty-one injected only two agents were found to be practically harmless. Moderate degrees of pulmonary congestion occurred with mild ether anesthesia used in connection with skin incisions and with normal saline (two out of four animals). In the particular experiment with ether here reported, the anesthetic was administered rather liberally, while with the remaining animals only a whiff of ether was used. The summary in Table 2 indicates that pulmonary congestion was uniformly present in all the experiments, indicating that in part, at least, the ether anesthesia was responsible. However, it is seen that in the experiments with other agents except normal saline, hemorrhage was a rather constant accompaniment of pulmonary congestion, indicating that these agents per se probably accentuated the congestion as a result of which hemorrhage was the natural sequel. Therefore, the effects of the ether anesthesia can be largely discounted. This is further supported by the absence of any demonstrable pulmonary and other changes whatsoever in the majority of animals injected with normal saline, also anesthesized with ether.

CONCLUSIONS

Mild ether anesthesia and the intravenous injection of saline are practically harmless to the circulatory and respiratory systems of guinea-pigs, but the remaining twenty-nine of the thirty-one agents studied were found to be distinctly harmful in varying degrees.

2. *Agents Whose Injurious Effects are Expressed Through the Respiratory System Chiefly*

Agar.—Of all the colloids that were tested agar was found to be the most injurious, and this was expressed principally through the respiratory system. As a rule, thrombi and conglutination of corpuscles were observed in the pulmonary capillaries. Aside from this, nothing abnormal in the circulation was observed. In a future communication it will be shown that agglutination of corpuscles by agar can also be reproduced in vitro, which, when taken together with thrombus formation in vivo, indicates that the primary mechanism of agar disturbances is associated with or due to its effect on the red blood corpuscles. This mechanism appears to be associated with the peculiar nature of agar, but not necessarily because of its colloidal nature. Somewhat different symptoms and effects were observed with other colloids, such as acacia, dextrin and starch. Agar appears to stand quite alone in the very prompt and striking pulmonary effects which it produces when injected intravenously.

The quantity of agar necessary to produce these profound effects and even death, is remarkably small, namely, about 0.014 to 0.05 mgm. per gram of body weight or about 0.014 to 0.025 per cent. concentration of agar in the blood of a 200 to 300-gram animal in our experiments. It was shown previously by Novy that the smaller the aggregates, such as are presumably obtained in agar sol-gel, the more profound the effects. In our experiments no special differences between agar sol, agar gel, and agar sol-gel were observed, and toxifying agar with rabbit's serum did not increase its toxicity in the two experiments that were performed. Fatalities occurred with the agar sol and agar sol-gel. For our purposes it sufficed to show that agar as an example of a typical colloid is definitely injurious in small quantities, also that this injury produced anaphylactoid phenomena, which outwardly, at least, that is, in a clinical way, resembled the alleged disturbances produced by the intravenous injection of arsphenamin and other agents, if not anaphylactic shock itself.

So far as the results of the injection experiments here reported are concerned, the marked inflation of the lungs produced could not be differentiated from the marked inflation of anaphylactic shock. In anticipation of results to be reported in subsequent papers it can be stated that the pulmonary inflation is not due to stimulation of smooth muscle by the agar. This was determined by perfusion of lungs, the treatment of both intact and surviving lungs with atropin, epinephrine and papaverin (to produce bronchial relaxation) and experiments with surviving intestine and uterus. The perfused lungs distended rapidly and there was a stoppage of the perfusion flow promptly. This would indicate peripheral stimulation of bronchial muscle. However, in the absence of relief by atropin and papverin of this effect, and the absence of uterine and intestinal stimulation, it is concluded that agar does not stimulate smooth muscle. There is no reason to believe that bronchial muscle is specifically stimulated by agar, or that agar is more powerful than histamin. In addition, the pulmonary vessels (arterioles and capillaries) of intact animals injected with agar sol-gel contained numerous thrombi, emboli and conglutinated corpuscles. The vessels of lungs perfused with agar sol-gel were loaded with agar emboli and thrombi and this caused the perfusion fluid to stop.

On the other hand, no emboli, thrombi or conglutinated corpuscles were demonstrable in perfused or intact lungs of anaphylactic shock, after peptone, certain serums and histamin. All of these agents were also active smooth muscle stimulants in other regions besides bronchi, namely, uterus and intestine. Pulmonary inflation occurred and this was relieved in intact lungs (except histamin) by atropin and epinephrin and in perfused lungs by atropin and papaverin. It is well known that epinephrin and atropin can relieve the bronchoconstriction of perfused lungs due to a variety of stimulants, but this is not true of agar. It is the occurrence of pulmonary emboli and thrombi with agar, resulting in passive bronchoconstriction (really compression of bronchi) that, we believe, is of the greatest signifiance in explaining the pulmonary distention and resultant phenomena. This at once separates the action of agar from true anaphylactic shock, and places all similarly acting agents into the same category with agar. It is deemed necessary to make this plain now, since this will facilitate and simplify the discussion of the remaining agents in this paper. That is, either an agent will act like agar producing passive broncho constriction and pulmonary inflation with the attendant symptoms of asphyxia, etc., or act partially like agar in which the effects are mixed with those of asphyxia, occurring primarily or secondarily, depending on the extent of damage to the circulation. A priori, therefore, it is obvious that the disturbances arising from the intravenous injection of various agents (arphenamin, acacia, etc.) may have been erroneously attributed to anaphylaxis and anaphylactic shock arising therefrom. This will be substantiated more fully in the discussions to follow and finally also confirmed in various ways in future publications.

Bile.—The single experiment performed with dog's bile indicates that it is distinctly injurious, producing a strong pulmonary inflation, thrombi and death, behaving in this respect not unlike agar. Of what importance this might be for the elucidation of the symptoms of jaundice is not known.

Human Serum.—The results obtained were similar to those commonly obtained with other toxic foreign serums such as beef serum previously studied by one of us.[9] The general absence of thrombi and the well known augmentor effects on smooth muscle suggest a different mechanism of action from agar.

Typhobacterin.—The results obtained in the one experiment that was performed agree entirely with the effects usually obtained after the injection of dead organisms, vaccines and similar preparations. The injection of larger organisms such as dead trypanosomes produces similar effects on intravenous injection (Novy). Whether the mechanism of action is different from, or similar to agar action as believed by Novy, can not be said at this time, since the subject could not be adequately investigated by us.

Conclusions.—The injury produced by the intravenous injection of the following agents is expressed through the respiratory system chiefly, producing definite anaphylactoid symptoms, and pulmonary distention, congestion, hemorrhages and thrombi without definite cardiac dilatation; agar sol (0.5 per cent.), agar gel (0.5 per cent.), agar sol-gel (1 : 4 and 1 : 6), dog's bile, human serum and typhobacterin. Pending further proof the mechanism of agar action may be stated to consist of passive bronchoconstriction with consequent phenomena of asphyxia. Consequently the disturbances of intravenous administration following the injection of various agents in unsensitized animals do not necessarily constitute true anaphylaxis or anaphylactic shock.

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[9] Karsner: Centralbl. f. Bakt. Parasiten. u. Infektionskr. **61**:247, 1911.

3. Agents Whose Injurious Effects are Expressed Mainly Through the Circulatory System

Althea (marshmallow).—According to Ono[10] althea is a strong agglutinator of corpuscles in vitro. In our injection experiments, fine pulmonary thrombi after the intravenous injection of althea extract (15 per cent.) were present in two out of three animals. Extracts of althea, even when fresh, are acid in reaction. This may explain the results in vitro, but in vivo the effects would depend on the quantity injected and available reserve alkali for neutralization. The dosage used was rather large and althea is also colloidal in nature. It is seen that pulmonary distention was generally absent, although symptoms were present, due no doubt to the injury to the circulation as indicated by cardiac dilatation, pulmonary congestion and hemorrhages which were present.

Arsphenamin.—It is claimed by Stokes[11] that arsphenamin is precipitated from its colloidal form in the circulation, and this or the precipitation of blood colloids evokes the "nitritoid crises" of arsphenamin poisoning which is a form of anaphylatic shock. The term "nitritoid crises" was introduced by Milian[12] because of the similarity of the symptoms to those from amyl nitrite. These consist principally of redness of face, dyspnea, feeling of anguish and distress, cough and precordial pain in human individuals after intravenous injection (Berman,[13] Beeson,[14] Hirano [15]). Berman explains the arphenamin dusturbances by precipitation occurring with sera of individuals exhibiting so-called "nitritoid crises." Although Berman claims to obtain precipitation in vitro with the sera of these individuals and not with those without symptoms, the precipitation or arsphenamin in the blood stream still remains to be demonstrated. We have observed frequently that insufficiently alkalinized solutions of arsphenamin will precipitate on the addition of 0.9 per cent. NaCl, but this does not occur if a slight excess of alkali is added. From their experimental work on dogs, Jackson and Smith concluded that arsphenamin raises the pulmonary arterial pressure and dilates the right heart, and this seemed to be associated with the alkalinity of arsphenamin. Pulmonary capillary obstruction such as by thrombi, emboli, etc., was not looked for by Jackson and Smith.[16] The anaphylactic explanation invoked by Stokes did not appear plausible in view of Auer's [17] work with arsphenamin on guinea-pigs. Hirano [18] claims a diminution in epinephrin content of the adrenals is responsible for the symptoms.

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[10] Ono: Acta Scholae Med. Univ. Imp. Kioto, **2**: 247, 1918.

[11] Stokes: J. A. M. A. **72**: 241, 1919.

[12] Milian: Bull. soc. francaise de dermat. et de syph. **23**: 520, 1912; **25**: 272, 436 1913.

In the experiments performed by us with therapeutic and even smaller doses of alkalinized arsphenamin prepared in the usual way, the guinea-pigs showed no gross pulmonary distention, and only microscopic distention in two out of the three animals. Conglutination thrombi were present in one animal. Congestion and hemorrhages in the lungs were marked in all animals. There was also definite cardiac dilatation. Taking all of the phenomena into consideration, together with our experience with other agents on about 160 animals, the results with the three animals injected indicate that arsphenamin, even in very small doses, injures the circulation, giving rise to symptoms of an anapyhlactoid nature. The perfusion experiments to be reported later also showed no pulmonary distention, indicating definitely that the arsphenamin reactions are not like anaphylaxis or anaphylactic shock. When precipitated solutions are injected, the disturbances might be like those from agar, and due to pulmonary emboli and thrombi. This would not necessarily occur because of the alleged colloidal form of arsphenamin.

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[13] Berman: Arch. Int. Med. **22:** 217, 1918.
[14] Beeson: Am. J. Syphilis, **3:** 129, 1919.
[15] Hirano: Kitasato Arch. Expt. Med. **3:** 1, 1919.
[16] Jackson and Smith: J. Pharm. Exp. Therap. **12:** 221, 1918.
[17] Auer: J. Exp. Med. **14:** 497, 1911.
[18] Hirano: Japanese Med. World, June 22, 1919.

This is supported by the results of our experiments with sodium arsenate, which is a crystalloid and produced practically the same phenomena as arsphenamin and neoarsphenamin. There is no reason to believe that sodium arsenate is transformed to a colloidal form in the circulation.

Collargol.—This is a silver-protein suspensoid colloid recommended for intravenous injection as an antiseptic and is claimed to be harmless. Different doses in different concentrations (within the therapeutic range in two animals) were injected into three guinea-pigs. All were distinctly harmed as indicated by the presence of definite symptoms. Two injections were fatal. These were the higher doses used. Two of the three animals showed the presence of pulmonary thrombi; absent or slight distention, congestion of variable degree and hemorrhage in only one animal. Definite cardiac dilatation was present in all. Collargol, therefore, is distinctly injurious and principally to the circulatory system, the claims of its advocates as to harmlessness to the contrary notwithstanding.

Congo Red.—This is another suspensoid colloid, differing from collargol chemically, but resembling it pharmacologically. The data in Table 1 indicate that congo red in small quantities is a distinctly harmful agent. Definite symptoms of respiratory distress were produced together with definite pulmonary distention in one, and slight distention in another out of the three animals injected; marked congestion in all and hemorrhage in one. Cardiac dilatation was uniformly present. Apparently, therefore, congo red injured chiefly the circulation, in the absence of pulmonary thrombi and emboli.

Dextrin.—Gross distention of the lungs after injection of dextrin was practically absent, but present microscopically in two out of the three animals treated. Marked pulmonary congestion was present in all and hemorrhage in two animals. This together with the cardiac dilatation indicates principal injury to the circulatory system which is quite sufficient to explain the symptoms of respiratory distress that were present.

Gelatin.—Gelatin has been used to some extent as a constituent of colloidal solutions intravenously for support of the circulation in shock, and in the treatment of hemorrhage. The objections to its use have been mainly the dangers of infection from imperfect sterilization. Other objections might be raised on the basis of results obtained by us with guinea-pigs.

The changes produced were not uniform. Symptoms of respiratory distress were present in the majority of animals; also moderate pulmonary distention microscopically, marked hemorrhages, conglutination thrombi (one animal) and definite cardiac dilatation. On the whole, it appears that the injury produced by gelatin is of circulatory origin, the pulmonary distention being rather variable and uncertain and perhaps depending on asphyxia as a result of the circulatory injury. It must be concluded that solutions of gelatin are not devoid of harmful effects when injected intravenously.

Inulin.—Inulin has been used by Nathan[2] and DeKruif and German[19] for the production of artificial anaphylatoxin, and apparently it is more effective when toxified with serum. In our experiments inulin alone in solution was used. Marked symptoms of respiratory distress were produced together with pulmonary congestion, but no distention. Thrombi were absent, and the heart was definitely dilated. The symptoms, therefore, appear to be associated with circulatory injury.

Phylacogen (Parke Davis Company).—The preparation called "Mixed Infection" was injected into three animals. It is highly recommended and advocated by the manufacturers for intravenous use in the treatment of different kinds of infections, even of doubtful etiology. The results obtained by us do not at all agree with the claims of harmlessness by the manufacturers.

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[19] DeKruif and German: J. Infec. Dis. **20**: 833, 1917.

No definite gross pulmonary distention was observed, but in two of the animals this was found to exist microscopically in marked and slight degrees. The presence of marked pulmonary congestion and hemorrhages was uniform throughout, and in one animal there was conglutination thrombosis. The symptoms of respiratory distress, which were present in all animals, appear to be principally of circulatory origin. It is obvious that phylacogen is distinctly injurious when used intravenously and might do more harm than good as an alleged therapeutic agent. This agrees with well founded suspicions of and clinical reports concerning philacogens as not only empirical but actually dangerous remedies.

Neoarsphenamin.—The effects of this arsenical (from three different sources) in therapeutic doses were similar to those of arsphenamin. The injury appears to be of circulatory origin as indicated by the uniform cardiac dilatation, marked pulmonary congestion and hemorrhages without definite gross or practically any microscopic distention. This is as would be expected with an arsenical agent.

Rabbit's Serum.—In the one experiment that was performed definite symptoms of respiratory distress were present, together with marked pulmonary congestion and slight hemorrhages, but no distention and thrombi. The heart was dilated, indicating that the serum was definitely harmful to the circulatory system. This serum, which was used to prepare toxified agar and acacia according to Novy's method, could account for a portion at least of the effects obtained with these preparations. The results with serum alone agree with the usual effects.

Sodium Arsenate.—This crystalloid arsenical was used as a control for the results obtained with arsphenamin, which is claimed by Daysz[7] to exist in a colloidal state in the circulation. However, the results being practically the same as with arsphenamin, and also neoarsphenamin, there is no reason to invoke any fancied peculiarity with arsphenamin. The effects of all the arsenicals tried are, no doubt, due to the arsenic though perhaps modified by the different forms in which the arsenic existed. Sorev[20] found that the primary mechanism of death in rabbits after the intravenous injection of colloid and crystalloid arsenicals was the same, namely, obstruction of pulmonary capillaries with the usual symptoms ot asphyxia. Launoy[21] concluded that death from colloidal and other arsenical compounds occurs in about the same way. Our results justify the conclusion that the symptoms after the injection of arsenical compounds, whatever the form of arsenic, are of circulatory origin.

Venarsen.—Sodium cacodylate is the principal constituent of this arsenical preparation. In the one experiment that was made, symptoms were absent, lungs collapsed, and there was marked pulmonary congestion with hemorrhage. Apparently, venarsen acts like other arsenicals, injuring the circulation principally.

Conclusions.—The following agents when injected intravenously were found to produce definite symptoms of respiratory distress together with pulmonary congestion and hemorrhages, but pulmonary distention and thrombi, as a rule, were absent: althea extract, arsphenamin, collargol, congo red, dextrin, gelatin, inulin, neoarsphenamin, phylacogen (mixed infection), rabbit's serum, sodium arsenate and venarsen. The injuries produced appear to be principally concerned with the circulatory system. The intravenous administration for therapeutic purposes of such agents as collargol and phylacogen, which still lack a scientific basis, is unjustified.

I. Agents Whose Injurious Effects Are Expressed Through the Circulatory and Respiratory Systems

Acasia.—The results with acacia, dialyzed acacia and acacia toxified with rabbit's serum were practically the same. The preparation and dosage of acacia were the same as is used therapeutically in the treatment of shock (traumatic, from hemorrhage, etc.). Symptoms of respiratory distress were present in all of the eight animals injected, and pulmonary distention was definite macroscopically and microscopically in the majority. Marked congestion of the lungs was present in all, and marked hemorrhages in five out of the eight animals. Thrombi in the pulmonary vessels were present in six out of the eight animals. Cardiac dilatation of variable degrees was present in all of the animals. The results obtained prove conclusively that acacia, when injected in doses and concentrations corresponding to those used in the treatment of traumatic shock, is definitely injurious. This is contrary to the results, pertaining chiefly to symptoms, reported by DeKruif,[22] but confirmative of the conclusions reached by Kruse,[23] and opinions held by many surgeons with extensive experiences in the treatment of shock during the recent war. In a later paper it will be shown that concentrations of acacia corresponding to those occurring in human blood agglutinate red blood corpuscles in vitro. This agrees with the property of acacia to form thrombi in vivo.

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[20] Sorev: Compt. rend. soc. biol. **81**: 57, 1918; Ibid. **81**: 563, 1918.

[21] Launoy: Compt. rend. soc. biol. **81**: 164, 1918.

Beef Serum.—Except for the presence of pulmonary thrombi, the results obtained with the single animal injected are confirmative of those previously obtained by one of us [9] and others, namely, that the intravenous injection of beef serum in guinea-pigs is distinctly injurious, involving both the respiratory and circulatory systems. The results as to thrombi would probably be different with a greater number of animals.

Glycogen.—When injected intravenously in the concentration of 0.5 per cent., glycogen is distinctly injurious. This is indicated by the presence of symptoms of respiratory distress, marked gross pulmonary distention and congestion and cardiac dilatation. Thrombi were not found after this colloid. The results of perfusion experiments to be reported later indicate no definite bronchoconstriction or augmentor action for other smooth muscle. The injury, therefore, appears to be mainly circulatory, the respiratory disturbance arising secondarily as a result of the circulatory injury. It is obvious why glycogen should not reach the circulation as such before conversion to the simpler carbohydrates.

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[22] DeKruif: Annals of Surgery, **69**: 297, 1919.
[23] Kruse: Am. J. Physiol. **40**: 137 (Proc.) 1919.

*Nuclein Solution.**—This is an aqueous extract of the wheat germ recommended for intravenous and hypodermic administration. Three animals were injected with doses ranging from 0.0015 to 0.009 c.c. per gram of body weight. The respiratory rate was increased in the majority, although no dyspnea was noticeable. Variable degrees of pulmonary distention, congestion and hemorrhages, and moderate cardiac dilatation were present in all. Pulmonary thrombi were absent. The results indicate that nuclein solution in the dosage used is distinctly injurious when injected intravenously.

Pancreatin.—With this a guinea-pig and a rabbit were used. The results obtained resemble those following the intravenous injection of peptone in guinea-pigs, as would be expected. Hemorrhages in the abdominal and pulmonary viscera occurred as claimed by Wago.[24]

Peptone.—The experiments with peptone, a protein colloid, served as controls in the same way as those with the different foreign serums for the non-protein colloids and other agents that were tested. The two animals that were injected responded in the usual way to peptone. The larger dose was promptly fatal, producing typical symptoms of respiratory distress, marked pulmonary inflation, and delayed coagulation of the blood as in anaphylactic shock. The smaller dose (0.019 mgm. per gram), which corresponded to that recommended by Nolf [25] for the treatment of certain infections and febrile conditions in human individuals, produced essentially the same effects as the larger dose, except death, and the vascular effects as indicated by marked pulmonary congestion and hemorrhages were more marked.

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* Supplied by the Abbott Laboratories, Chicago.

24. Wago: Arch. Int. Med. **23:** 251, 1919.

25. Nolf: J. A. M. A. **72:** 1901, 1919.

The heroic effects, which small doses of peptone are known to produce in human individuals, and the fact that they are distinctly injurious and that larger doses may be fatal should enjoin caution in the promiscuous and careless application of this kind of therapy. It is interesting to note that pulmonary thrombi are absent, as a rule, after the injection of peptone, foreign serums and in truly anaphylactic animals, indicating that peptone, foreign serums and histamin produce their effects in a different way from the various non-protein colloids and similar agents reported in this paper.

*Pollen Extracts.**—These were tested because of their plant protein nature and their common use in the treatment of hay fever. While they are not recommended for therapeutic use intravenously, it is conceivable that enough of these may be absorbed when administered hypodermically or applied on the mucous surfaces. Five animals were injected with extracts prepared from ragweed, mixtures of grasses used for spring hay fever and mixtures used for fall hay fever. The results with the different extracts were about the same.

Symptoms of respiratory distress as a rule were present, but gross pulmonary distention was variable, and microscopically occurred only in two out of the five animals. Marked pulmonary congestion was uniform throughout; hemorrhages occurred in the majority [4] and conglutination in two animals. The heart was generally dilated. These results indicate injury to the circulatory system principally, the symptoms of respiratory disturbance occurring secondarily as a result of the circulatory injury, and perhaps of asphyxial origin. The conclusion that pollen extracts when injected into (or reaching) the circulation are distinctly injurious is justified. Whether any such quantities could reach the circulation as were used in our experiments and when administered by routes other than intravenous is not known.

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* Supplied by H. K. Mulford Company, Philadelphia.

Starch.—Merck's soluble starch (2 per cent. and 6 per cent.) was injected into three animals. All were definitely and markedly injured as indicated by the presence of symptoms of respiratory distress, moderate distention of the lungs together with marked pulmonary hemorrhages and definite congestion and definite cardiac dilatation. The solutions in each case were filtered and resemble in appearance those of acacia. However, no pulmonary thrombi were observed. Okasaki [26] reports the presence of starch granules in the pulmonary capillaries after intravenous injection in rabbits. Our results are sufficient to indicate that such a colloid as starch, non-protein in character and otherwise harmless, when injected intravenously is definitely harmful, injuring the circulatory system principally, the symptoms of respiratory distress occurring secondarily.

Conclusions.—The intravenous injection of the following agents injures the circulatory and respiratory systems as indicated by the presence of anaphylactoid symptoms, pulmonary distention, congestion and hemorrhage together with cardiac dilatation: acacia, plain dialyzed and toxified rabbit's serum, beef serum, glycogen, pancreatin, peptone (large and very small doses), pollen extracts and starch. Thrombi in pulmonary vessels occurred after the injection of acacia in therapeutic doses and concentrations, and pollen extracts. The indiscriminate and careless administration of acacia and peptone intravenously should be enjoined with caution.

5. *Dangers of Intravenous Medication.*

The results of the experiments in this investigation are sufficiently conclusive against the promiscuous, careless and unwarranted use of the intravenous method of administering drugs as a routine measure for therapeutic purposes. The disadvantages of damage to important systems like the circulation and respiration certainly outweigh the advantages that are to be gained from the administration of remedies of doubtful efficacy and those whose actions are produced equally or about as readily by other methods. Notable exceptions to this are such well known and tried remedies as strophanthin and arsphenamin.

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[26] Okasaki: Chem. Absts. **13**: 45, 1919.

The dangerousness of certain remedies can be sufficiently predicted from their pharmacological action and toxicity. On the other hand such colloidal agents as agar, acacia, gelatin, glycogen, starch and dextrin which possess no demonstrable pharmacological actions and are non-toxic in the usual sense of the word, that is, because of their inherent chemical properties, and are, in fact, practically inert, yet when injected intravenously into animals are distinctly injurious. This certainly could not be predicted from our knowledge of their chemical and pharmacological properties; and emphasizes the fact that without actual trial, speculation may lead to erroneous conclusions. This should be impressed with an agent like acacia, which, although already suspected of deleterious qualities in clinical practice, was nevertheless advocated with considerable zeal. How much actually can be gained by the intravenous injection of acacia in shock, and similar conditions, in view of its harmful qualities, granting even that its use is dictated by certain clinical experience or is well established on physiological grounds, may indeed be doubted. Its use on a physiological basis is doubted by Prof. G. N. Stewart.[27]

What has been said of acacia also pertains to peptone, which is being advocated as a therapeutic measure. With proteins the deleterious effects are better appreciated. With certain other agents which partake of both the colloidal and protein nature, such for instance as collargol, the possibilities of harm should be even more patent. This is illustrated by the action of congo red. which is a suspensoid colloid similar in type physically to the silver portion collargol, though non-protein in nature. Further illustrations are found in the various hydrophilic colloids discussed above.

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[27] Stewart, G. N.: Amer. J. Physiol. **60**: 233, 1919.

Concerning the remaining agents, which are used or advocated for use therapeutically, namely, arsphenamin, neoarsphenamin, venarsen, phylacogen, nuclein solution and typhobacterin, perhaps enough has been said in the text. The unscientific basis for the phylacogens and even nuclein has been sufficiently pointed out. The intravenous injection of phylacogen, at least, in large or small doses is decidedly unjustifiable. Typhobacterin and other vaccines are used hypodermically in their proper rôle, and the extensive successful use of typhoid and some other vaccines testifies adequately to their relative harmlessness when used in this way. The heroic effects occurring after the intravenous injection of typhoid vaccine and foreign proteins as practiced by some in the treatment of rheumatic fever, etc., does not appear so well justified.

Concerning the organic arsenicals, like arsphenamin, neoarsphenamin and venarsen, the results obtained were about the same as with sodium arsenate, an inorganic arsenical. The toxicity of these agents is generally admitted, and our experiments indicate that even in very small doses the action of arsenic on the circulatory system is still evident. The invocations of colloidal and other transformations for arsphenamin in the circulation to explain the so-called "nitritoid crises" appear to be subtle distinctions that are unnecessary. That these disturbances appear to be concerned to a considerable extent with the method of preparing arsphenamin solutions (insufficient alkali, precipitation, etc.) is quite evident from the detailed and careful instructions for this by the manufacturers of these preparations and in part, at least, to the arsenic, but not to true anaphylaxis or anaphylactic shock, as indicated by the results of our experiments and also of others. This brings us to the consideration of the relation of our results to anaphylaxis.

Conclusion.—The results obtained are decidedly against the promiscuous and unwarranted use of the intravenous method of administering drugs as a routine therapeutic measure, particularly for new and untried remedies or those of doubtful efficacy, and even those which chemically and pharmacologically appear to be inert or inactive.

6. Relation to True Anaphylaxis and Anaphylactic Shock

It is unnecessary here to discuss the merits and demerits of the numerous theories of anaphylaxis and anaphylactic shock. A summary of most of these may be found in a paper by Dale,[28] and some of these have been recently discussed in a monograph by Besredka.[29] A close scrutiny of the following reveals that these have no points in common with such colloidal agents as agar, starch, dextrin, acacia, etc., or are sufficiently objectionable on other grounds, abounding in postulated substances and mechanisms; apotoxin theory of Richet,[30] anaphylatoxin theory of Friedberger,[31] physical theory of Doerr and Moldovan,[32] ferment theories of Vaughan [33] and of Jobling and Petersen,[34] phyical nervous theory of Besredka [29] and peptone theory of Biedl and Kraus.[35] The following theories, or explanations, however, require some consideration because they possess certain points of contact with or are based on the type of agents studied by us.

Bronchial theory of Auer and Lewis.[36]—The marked bronchoconstriction which occurs prevents egress of air during the expiratory phase and the lungs remain distended, the animal dying of asphyxial symptoms and constituting those of anaphylactic shock. Shock and death, though not necessarily all of the symptoms, are prevented by atropine. The bronchial muscle itself (after vagus degeneration) is hyperexcitable.

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28. Dale: J. Pharm. Exp. Therap. **4**: 167, 1913.
29. Besredka: Anaphylaxie et Antianaphylaxie, Masson et Cie, Paris, 1917.
30. Richet: L'Anaphylaxie, Paris, 1912.
31. Friedberger: Ztschr. f. Immunitätsf. **4**: 636, 1910.
32. Doerr and Moldovan: Biochem. Ztschr. **41**: 27, 1912.
33. Vaughan: J. Inf. Dis. **4**: 476, 1907.
34. Jobling and Petersen: J. Exp. Med. **22**: 468, 1914.
35. Biedl and Kraus: Wein. klin. Wchnschr. **22**: 363, 1910.
36. Auer and Lewis: J. A. M. A. **53**: 458, 1909; J. Exp. Med. **12**: 151, 1910.

Schultz[37] and Dale[28] showed that the intestinal and uterine muscles of sensitized guinea-pigs share the increased excitability. These phenomena are so constant in truly anaphylactic animals that no investigation is complete without giving them due consideration. In fact, it may be said that the pulmonary phenomena are *the* one set of phenomena clearly and easily recognizable in guinea-pigs occurring both in true anaphylactic shock and the disturbances from the intravenous injection of several foreign agents, notably agar, congo red, acacia and others studied by us. However, agar alone causes inflation of the perfused lung. This will be referred to again later.

Taraxy Theory of Novy and DeKruif.[38]—This is intended to correlate and explain the effects ("nonspecific anaphylactic shock") produced by the intravenous injection of a variety of agents, namely, bacteria, trypanosmes, organ cells, organ extracts, peptone, agar, starch, inulin, kaolin, silicic acid, barium sulphate, etc. A matrix of the poison in the circulating plasma and serum is presupposed and this is called "taraxigen" (from "taraxy," meaning disturbed state or condition from the poison), and it stautomeric poisonous form is "tarxin." The transformation of fibrinogen to fibrin may be likened to the change supposed to take place. A sudden formation of, or too much, "taraxin" brings the explosive effects such as the reactions to foreign agents, and, it is suggested, even the symptoms of eclampsia. Chronic "taraxy" or anaphylactic poisoning is believed to exist in arsphenamin poisoning. The administration of bicarbonate is advocated as a treatment for "taraxies." In specific anaphylactic shock the inducing substance is formed by the interaction of antigen with its antibody. It does not appear to us that the introduction of new terminology and further assumptions will materially assist in the explanation of so many diverse conditions.

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[37] Schultz: J. Pharm. Exp. Therap. **1**: 549, 1910; Hyg. Lab. Bull., U. S. P. H. S., No. 80, 1912.

[38] Novy and DeKruif: J. A. M. A. **68**: 1524, 1917.

Von Behring's Platelet Theory.[39]—The symptoms of anaphylactic shock are ascribed to cerebral capillary embolisms produced by the clumping of platelets. Some disturbance in their medium or themselves causes the platelets to clump and consequently obstruct the smallest blood vessels. Dale and Laidlaw[40] recently observed the abnormal abundance and clumps of agglutinated platelets in the blood of cats injected with histamin. The presence of thrombi and conglutination of corpuscles in the pulmonary vessels was commonly observed by us with the majority of the agents injected, but only in one case was there a precipitation of platelets noticed, though, of course, there would be no justification for assuming the liberation of histamin with our agents.

Histamin or Histamin-like Substances.—The recent demonstration of the presence of histamin in peptone (Witte) by Abel and Kubota[41] would appear to fortify the peptone theory of Biedl and Kraus[35] and a view formerly held by Dale and Laidlaw.[42] However, the presence and quick elaboration of a peptone or histamin-like substance still remain to be demonstrated in the anaphylactic shock of guinea-pigs, and its elaboration by non-protein agents like agar, dextrin, etc., would be still more doubtful unless any kind of endothelial injury as suggested by Dale and Laidlaw might do so. In that case the various agents studied by us and anaphylaxis might be shown to enjoy a mutual relationship. However, at present the facts are too limited to justify any such generalization.

Pseudoanaphylaxis. — Kritchewsky[6] elaborated a theory on the basis of a parallelism between agglutinating power and ability to cause shock, but this was based only on the power of cotyledon juice to agglutinate bacteria and corpuscles, and precipitate serum. Besides bronchoconstriction and vascular spasm, a type of anaphylactic reaction due to a plugging of pulmonary blood vessels with thrombi and agglutinated corpuscle masses is recognized by Manwaring and Crowe.[43] This is called "pseudoanaphylaxis." The reactions with the different agents described by us come closer to this than to any other designation, if anything of the sort is needed at all.

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[39] von Behring: Deutsch. med. Wchnschr. **40**, No. 41, 1914.
[40] Dale and Laidlaw: J. Physiol. **52**: 355, 1919.
[41] Abel and Kubota: J. Pharm. Exp. Therap. **13**: 243, 1919.
[42] Dale and Laidlaw: J. Physiol. **41**: 318, 1910.

It is seen that some of these theories run along similar channels, but none of them satisfactorily or adequately explain the symptoms and disturbances produced by the injection of the non-protein colloids and other agents in unsensitized animals as reported in this paper. This is probably equally true of anaphylaxis and anaphylactic shock. To recapitulate:

1. Agar, acacia, dextrin, glycogen, starch, inulin and similar colloids are inert and practically non toxic, as far as their inherent chemical properties are concerned.

2. These colloids are not known to elaborate precipitins or toxins or cause precipitation of the blood. On the contrary they could conceivably adsorb toxins and limit their action. Concerning the rôle of agglutination of corpuscles this will be discussed in a subsequent publication.

3. They are non-protein in character.

4. They possess no known irritant or augmentor effects on smooth muscle (bronchi, intestine and uterus). Proof of this will be submitted later.

5. They are not ferments nor act as such, neither are they known to disturb ferment-antiferment balance. Their action is too rapid. 5a. They do not alter the coagulation of blood.

6. They have no known effects on nerve centers, trunks, endings, ganglia, myoneural junctions, etc., directly.

7. The effect of organic arsenicals like arsphenamin, etc., can be adequately explained by the well known actions of arsenic on the circulation, and imperfections in the technique of administration.

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[43] Manwaring and Crowe: Proc. oc. Exp. Biol. Med. **14:** 100, 1917.

8. The fact that the majority (twenty out of thirty-one) of the agents studied by us can be classified as having injured the circulation or the circulation and respiration, the latter being involved as a result of circulatory injury, indicates that no other mechanism such as anaphylaxis or anaphylactic shock need be invoked.

9. Furthermore, no previous treatment or sensitization of the animals with these agents is necessary. Sensitization is a sine qua non requirement for the true anaphylactic shock, requiring at least eight days whatever the dosage of serum according to Besredka. Hence it does not appear that desensitization or anti-anaphylaxis can be invoked or regarded as part of the mechanism, a condition Besredka is justified in raising against claims of relationship between anaphylaxis and disturbances produced by various foreign agents (starch, kaolin, agar, etc.).

10. It has even been doubted if these theories explain the responses to injections of peptone and heterologous serums, whose actions present many similarities to, but lack some of the characteristics of anaphylactic poisoning (Loewit,[44] Karsner[45]).

Conclusion.—When taken together with the explanations supplemented throughout the text, our results indicate that it is quite erroneous to regard the disturbances produced by the intravenous injection of sundry agents in the same category with true anaphylaxis, or bearing any causal relationship to it whatsoever or vice versa.

7. *Specific Gravity of Collapsed and Distended Lungs*

This is intended as a supplement to the work reported in this paper. Sometimes it is difficult to correctly interpret distention or inflation of the lungs in anaphylactic shock. It is well known that collapsed and even edematous and hemorrhagic and, of course, distended lungs of guinea-pigs will generally float on water. Therefore, some lighter medium was sought by which it might be possible to differentiate collapsed and distended lungs. Ordinary ethyl ether of specific gravity ranging from 0.711 to 0.716 out of a number of fluids of low specific gravity was found to answer the purpose very well. This is indicated by the results with 114 different lungs of guinea-pigs used in our experiments and recorded in Table 3.

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[44] Loewit: Arch. f. Exp. Path. Pharm. **73:** 1, 1913.
[45] Karsner: Ztschr. f. Immunitätsf. u. exp. Therap. **14:** 81, 1912.

Observations on 107 different lungs indicated that when their specific gravity was increased with the degree of congestion encountered these were all lighter than water, but heavier than ether when collapsed and congested (thirty-one out of thirty-two lungs). When even a moderate degree of distention accompanied by moderate congestion was present, about 77 per cent. of seventy-four lungs tested floated on ether, and all or 100 per cent. of the five tested floated when distention alone was present. Two collapsed lungs without congestion and hemorrhages sank in ether and floated on water. That is, a lung partially or markedly distended, in other words, partially or wholly anaphylactic, is lighter (floats) than ether, while a lung that is simply congested, or collapsed and congested (not anaphylactic), is heavier than ether. We found this test of some value in interpreting the results of our work.

TABLE 3.—SPECIFIC GRAVITY OF COLLAPSED LUNGS

Condition of Lungs	Number of Lungs Tested	Lighter Than Ether (Float)		Heavier Than Ether (Sink)		Lighter Than Water	
		Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
Distention alone	5	5	100	..	...	5	100
Collapse alone	2	..	...	2	100	2	100
Collapse and congestion	32	1	3	31	97	29*	100
Distention and congestion	74*	57	77	17	23	75	100
Total number lungs tested	114†						

* No more lungs were tested.
† One more added from column "Lighter than water."

Conclusion.—Ether immersion appears to be a simple test for distinguishing collapsed and congested lungs from distended and congested lungs possessing a moderate to a strong degree of distention.

IV. CONCLUSIONS

1. Mild ether anesthesia and the intravenous injection of normal saline are practically harmless to the circulatory and respiratory systems of guinea-pigs, but the remaining twenty-nine of the thirty-one agents studied were found to be distinctly harmful in varying degrees.

2. The injury produced by the intravenous injection of the following agents is expressed through the respiratory system chiefly, producing definite anaphylactoid symptoms, and pulmonary distention, congestion, hemorrhages and thrombi without definite cardiac dilatation: agar sol (0.5 per cent.), agar gel (0.5 per cent.), agar sol-gel (1 : 4 and 1 : 6), dog's bile, human serum and typhobacterin. Pending further proof, the mechanism of agar action may be stated to consist of passive bronchoconstriction (really compression of bronchi) with consequent phenomena of asphyxia. Consequently, the disturbances of intravenous administration following the injection of various agents in unsensitized animals do not necessarily constitute true anaphylaxis or anaphylactic shock.

3. The following agents when injected intravenously were found to produce definite symptoms of respiratory distress together with pulmonary congestion and hemorrhages, but, as a rule, no distention and thrombi: althea extract, arsphenamin, collargol, congo red, dextrin, gelatin, inulin, neoarsphenamin, phylacogen (mixed infection), rabbit's serum, sodium arsenate and venarsen. The injuries produced appear to be principally concerned with the circulatory system. The intravenous administration for therapeutic purposes of such agents as collargol and phylacogen, which still lack a scientific basis, is unjustified.

4. The intravenous injection of the following agents injures the circulatory and respiratory systems as indicated by the presence of anaphylactoid symptoms, pulmonary distention, congestion and hemorrhage together with cardiac dilatation: acacia, plain, dialyzed and tox-

ified with rabbit's serum, beef serum, glycogen, nuclein solution, pancreatin, peptone (large and very small doses), pollen extracts and starch. Thrombi in pulmonary vessels occurred after the injection of acacia in therapeutic doses and concentrations, and pollen extracts. The indiscriminate and careless administration of acacia and peptone intravenously should be enjoined with caution.

5. The results obtained are decidedly against the promiscuous and unwarranted use of the intravenous method of administering drugs as a routine therapeutic measure, particularly for new and untried remedies or those of doubtful efficacy, and even those which chemically and pharmacologically appear to be inert or inactive.

6. When taken together with the explanations supplemented throughout the text, our results indicate that it is quite erroneous to regard the disturbances produced by the intravenous injection of sundry agents in the same category with true anaphylaxis or bearing any causal relation to it whatsoever, or vice versa.

7. Ether immersion appears to be a simple test for distinguishing collapsed and congested lungs from distended and congested lungs possessing a moderate to a strong degree of distention.

Our thanks are due to Mr. Joseph Fetterman of the Third Year Class for valuable assistance in a portion of the work.

A COMPARISON OF THE PROPHYLACTIC EFFECTS OF ATROPIN AND EPINEPHRIN

IN ANAPHYLATIC SHOCK AND ANAPHYLACTOID PHENOMENA FROM VARIOUS COLLOIDS AND ARSPHENAMIN *

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I. INTRODUCTION

The results previously reported by us[1] with the intravenous injection of agar and other non-protein colloids and also the organic arsenicals, including arsphenamin, into guinea-pigs indicated that these bear no relationship to anaphylaxis or anaphylactic shock. Concerning agar and some other agents further proof is necessary to establish whether or not an actual stimulation of the bronchi exists in the accompanying pulmonary distention. This could be obtained in part, at least, by the use of atropin and epinephrin as bronchodialators in the prophylactic treatment of the distention.

That is, if the distention is due to active bronchoconstriction, such as in anaphylactic shock, this should be prevented or relieved by atropin as originally shown by Auer.[2] Atropin also antagonizes the bronchoconstrictor action of peptone in intact guinea-pigs (Biedl

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1. Hanzlik and Karsner: J. Pharm. Exp. Therap. **14:** 379, 1920.
2. Auer: Am. J. Physiol. **26:** 439, 1910.

and Kraus,[3] and of both peptone and histamin in perfused lungs (Baehr and Pick[4]). The dose of atropin bears a quantitative relationship to the size of the intoxicating dose of protein.[5]

With epinephrin the effects are more complicated and not so certain, depending in part on the functional state of the bronchiolar musculature (Januschke and Pollak[6]) and in part on the time of administration, since the action is fleeting. Januschke and Pollak found that epinephrin relaxes the untreated bronchi somewhat and the greatest bronchodilator action was obtained when the bronchi were previously constricted by peptone and muscarin, but not by histamin and ergotoxin. Epinephrin also relieves the bronchoconstriction by peptone in guinea-pigs (Biedl and Kraus[3]) and that produced by peptone and histamin in perfused lungs when high concentrations are used (Baehr and Pick[4]).

It is apparently on the basis of their bronchodilator effects (perhaps circulatory, in part) that the use of epinephrin is recommended by Milian,[7] Beeson[8] and Hirano[9] and atropin by Stokes[10] in the treatment of the "nitritoid crises" of arsphenamin poisoning, which is regarded by Stokes as a form of anaphylaxis. Nolf[11] advises the use of epinephrin in connection with the disturbances accompanying proteose therapy. Whatever the clinical success with the use of these drugs, experimentally, at least, the basis has not been clearly established. It does not necesarily follow that whatever beneficial effects they may have are due to their action on the bronchioles.

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3. Biedl and Kraus: Zent. f. Physiol. **24:** 258, 1910.
4. Baehr and Pick: Arch. f. exp. Path. Pharm. **74:** 65, 1913.
5. Karsner and Nutt: J. A. M. A. **57:** 1023, 1911.
6. Januschke and Pollak: Arch. f. exp. Path. Pharm. **66:** 205, 1911.
7. Milian: Bull. Soc. francaise de dermat. et de syph. **23:** 520, 1912; **24:** 272, 1913.
8. Beeson: Am. J. Syphilis **3:** 129, 1919.
9. Hirano: Kitasato Arch. Exp. Med. **72:** 241; Jap. Med. World, June 22, 1919.
10. Stokes: J. A. M. A. **72:** 241, 1919.
11. Nolf: J. A. M. A. **72:** 1901, 1919.

We have, therefore, attempted to test out this and also other features of anaphylactoid reactions. The main objects of the work may be stated as follows: (1) Prophylactic treatment of disturbances accompanying the administration of arsphenamin, peptone and non-protein colloids experimentally in guinea-pigs by means of atropin and epinephrin; (2) elucidation of the mechanism of pulmonary distention with agar by means of these agents; (3) treatment of anaphylactic shock in serumsensitized guinea-pigs with epinephrin, for, so far as we know, this has not been tried. In dogs Pelz and Jackson[12] found epinephrin to relax the constricted bronchi in anaphylactic shock if injected early.

II. METHODS

Because of the pulmonary effects, which we were chiefly investigating, guinea-pigs were chosen as the most suitable animals. These were injected intravenously in the same way as described in the previous paper. The various agents used to produce the respiratory disturbances were prepared in exactly the same way also. These served as controls for the experiments with atropin and epinephrin reported in this paper. For description of the results with these, as well as the methods used, the previous paper[1] should be consulted. It is only necessary to describe here the method of administering atropin and epinephrin.

Atropin sulphate (0.1 per cent.) was always injected intravenously and allowed to act about five minutes before the administration of the agent producing anaphylactoid symptoms and in the dosage of about 1 mgm. per 100 grams of animal. The usual effects of increased respiratory rate and hyperexcitability were observed.

Epinephrin was used intravenously in two ways; (1) Immediately preceding, and (2) together with the agent to be observed. The dosage was that which produces a definite rise of blood pressure in dogs, namely, 0.5 c.c. of 1 : 10,000 per kilo or 0.0005 c.c. of 1 : 10,000 per gram of guinea-pig. Prompt effects were produced as indicated by the marked increase in respiratory and pulse rate. The following agents were studied: arsphenamin, agar, dextrin, congo red, peptone, beef serum, typhobacterin, acacia, pollen extract, starch, althae and serum-sensitized animals.

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[12] Pelz and Jackson: J. Pharm. Exp. Therap. **11**: 173, 1918.

III. DISCUSSION OF RESULTS

The data pertaining to individual experiments with the different agents are presented in Tables 1 and 2. The descriptions of the controls, that is, effects of different agents without atropin and epinephrin as well as normal saline, have been presented in a previous paper (1) and are omitted in this paper to save space. Instead, a comparison of all the changes obtained with the different agents in the present series has been made with those of the controls and the whole is conveniently summarized in Table 3.

1. Prevention by Atropin

From the summary in Table 3, it is seen that atropine in the dosage of 0.01 mgm. per gram of animal, equvalent to 600 to 1,000 times the average therapeutic dose for man, acts favorably as a preventive for the effects of beef serum and peptone. Complete prevention as to symptoms, necropsy and microscopic changes were obtained with beef serum in one animal and somewhat less in another. The usual effects of a small dose of peptone, equivalent to the therapeutic dose recommended by Nolf [11] in the treatment of infectious and febrile conditions, were completely prevented by atropin. The protection was not so complete with a dose once as large; but death, at least, was prevented.

The partial protection afforded by atropin against the effects of agar sol, agar sol-gel, arsphenamin, congo red, serum-sensitization and typhobacterin was concerned principally with diminution in the severity of symptoms. Fatalities from agar sol, agar sol-gel and the anaphylactic (serum-sensitized) animals were prevented. So far as necropsy and microscopic changes are concerned there was practically no difference from the controls. The partial prevention from the effects of beef serum and peptone have been discussed above.

Concerning agar sol-gel, the interpretation of "partial prevention" by atropin is perhaps liberal. That is, the controls received a small dosage, while in the present experiments the dosage was practically fatal as shown by the results of Novy and DeKruif [12] and our own experiments with agar sol-gel and epinephrin, in which fatalities occurred with the same dosage. For this reason, the results with atropin are interpreted as indicating some protection (as to fatality), and there is certainly no doubt about this with agar sol. However, the atropin did not prevent the general effects of agar sol-gel, namely, symptoms of respiratory distress, marked pulmonary distention, hemorrhages and thrombi, although the thrombi were somewhat less conspicuous. These experiments, therefore, so far as the mechanism of agar sol-gel action is concerned, are indecisive as originally hoped for. The comparisons which we hoped to obtain with various smooth muscle (bronchiolar) stimulants, namely, peptone, beef-serum and serum-sensitized animals, are too variable to permit the free use of these, although the tendency is favorable with beef serum and peptone. That is, complete prevention was obtained with these augmentors of smooth muscle (bronchiolar, etc.) in some animals and this was certainly more than was obtained with agar, indicating that the mechanism of agar action is different from peptone and simlar augmentors of plain muscle. This would mean that the anaphylactoid effects of agar are not like or partake of true anaphylactic shock. However, this statement is made reservedly considering the variability of results obtained with the smooth muscle stimulants in this paper.

This is even more true of the results with epinephrin and agar sol-gel presently to be discussed. Because of this, and a certain amount of variability in the response of guinea-pigs to the various agents that have been tried, including agar, it is manifestly impossible

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13. Novy and DeKruif: J. Inf. Dis. **20**: 536, 1917.

TABLE 1.—TREATMENT OF ANAPHYLACTOID PHENOMENA FROM VARIOUS AGENTS WITH ATROPIN

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm. Guinea-Pig	Principal Symptoms	Fate of Animal Minutes	Necropsy	Microscopic Examination of Lungs
				Dextrin (6 per cent.)			
50	250	tropin 3 mg. 6 per cent. dextrin 3 c.c......	0.012 mg. 0.61 mg.	Slight dyspnea, otherwise normal	Killed, 30½	Marked pulmonary distention and minute hemorrhages, slight cardiac and abdominal congestion	Marked distention, marked congestion, marked hemorrhage
111	500	Atropin 8 mg. Dextrin 3 c.c. of 10 per cent.	0.016 mg. 0.6 mg.	Marked increase in respiration; restlessness	Killed, 52	Marked pulmonary distention and congestion and slight hemorrhages; very marked cardiac dilatation; moderate abdominal congestion; blood clotted in 4 minutes	Moderate distention, marked congestion, slight hemorrhage
				Agar sol (0.5 per cent.)			
55	530	Atropin 3 mg. 3.0 c.c. of 0.5 per cent. agar	0.0099 mg. 0.046 mg.	Dyspnea; marked restlessness; cyanosis marked; depression marked; respiration slow	Killed, 31	Lungs distended and congested; marked cardiac dilatation, abdominal congestion	Moderate distention, moderate congestion, slight hemorrhage, marked thrombosis
				Agar sol gel (1 :6)			
60	230	Atropin 3 mg. 5 c.c. agar....	0.013 mg. 0.018 mg.	Dyspnea; respiration slow...	Killed, 30	Lungs distended, hemorrhages, no edema; slight cardiac dilatation, slight abdominal congestion	Marked distention, marked congestion, marked hemorrhage
97	430	Atropin 6 mg. 5 c.c. agar....	0.014 mg. 0.01 mg.	Marked dyspnea; respiration increased; depression marked; recovery from symptoms	Killed, 42	Marked pulmonary distention, congestion and hemorrhages; marked cardiac dilatation, abdominal congestion and gastric submucus hemorrhages; rapid blood coagulation	Moderate distention, marked congestion, slight hemorrhage, marked thrombosis
98	365	Atropin 6 mg. Agar 8 c.c. ...	0.017 mg. 0.019 mg.	Markedly increased respiration; no dyspnea	Killed, 33	Very marked pulmonary distention, hemorrhage and congestion; moderate cardiac dilatation; slight abdominal congestion; blood clots rapidly	Moderate distention, marked congestion, marked hemorrhage
92	250	Atropin 4 mg. Agar 5 c.c. ...	0.016 mg. 0.017 mg.	Marked dyspnea; increased respiration; restlessness at first, later amelioration of all symptoms	Killed, 33	Moderate pulmonary distention, hemorrhage and congestion; cardiac dilatation; abdominal congestion; blood clots rapidly	Moderate distention, marked congestion

				Congo red (1 per cent.)			
118	330	Atropin 4 mg. Congo red 3 c.c.	0.0091 mg. 0.091 mg.	Increased respiration; excitable; quiet	Killed, 43	Lungs collapsed and congested; no hemorrhages; abdominal congestion; slight cardiac dilatation; blood clotted in 4 minutes	Marked congestion, slight hemorrhage
156	470	Atropin 5 mg. Congo red 3 c.c.	0.0107 mg. 0.062 mg.	Increased respiration; later marked depression	Fatal, 20	Lungs collapsed and markedly congested; marked cardiac dilatation and abdominal congestion	Marked congestion, slight local distention
				Horse serum sensitization			
48	200	Atropin 3 mg. Serum 0.5 c.c., diluted to 3 c.c.	0.015 mg. 0.0025 c.c.	Marked increase in respiratory rate; dyspnea (?); increased motor excitability	Killed, 37	Slightly distended lungs, pneumonia right base; slight cardiac and abdominal congestion; 20th day sensitization	Moderate distention, moderate congestion
125	490	Atropin 5 mg. Serum 0.8 c.c. ..	0.0102 mg. 0.0017 mg.	Increased respiration; restlessness; no dyspnea	Killed, 42	Considerable pulmonary distention, marked congestion, no hemorrhages; moderate cardiac dilatation, no abdominal congestion; blood clotted in 4 minutes	Moderate distention, moderate congestion
				Beef serum			
117	450	Atropin 5 mg. Beef serum 2 c.c.	0.011 mg. 0.0044 c.c.	Increased respiration	Killed, 30	Moderate pulmonary distention; slight hemorrhage and moderate congestion; moderate cardiac dilatation and abdominal congestion; blood clotted in 3 minutes	Marked distention, marked congestion; marked hemorrhage
159	470	Atropin 5 mg. Beef serum 2 c.c.	0.0106 mg. 0.0042 c.c.	Restlessness	Killed, 43	Lungs practically collapsed, congested and hemorrhages; no cardiac dilatation and abdominal congestion	Slight congestion; slight local distention, practically normal

TABLE 1.--TREATMENT OF ANAPHYLACTOID PHENOMENA FROM VARIOUS AGENTS WITH ATROPIN.—Continued

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm. Guinea-Pig	Principal Symptoms	Fate of Animal Minutes	Necropsy	Microscopic Examination of Lungs
				Peptone (10 per cent.)			
112	540	Atropin 6 mg. Peptone 0.2 c.c.	0.011 mg. 0.037 mg.	Increased respiration; restlessness	Killed, 35	Pulmonary distention; small hemorrhages and moderate congestion; slight cardiac dilatation and moderate abdominal congestion; blood clots in 2 minutes	Moderate distention, marked congestion
158	280	Atropin 3 mg. Peptone 0.016 c.c.	0.011 mg. 0.0058 mg.	Restlessness; rerspiration increased	Killed,57	Lungs collapsed and congested with few hemorrhages; moderate cardiac dilatation	Slight congestion, slight local distention, practically normal
				Soluble starch (6 per cent.)			
119	520	Atropin 5 mg. Starch 3 c.c. ...	0.0096 mg. 0.34 mg.	Increased respiration, and dyspnea moderate	Killed, 44	Marked pulmonary distention, slight congestion and hemorrhages; slight cardiac dilatation and abdominal congestion; blood clotted in 3 minutes	Marked congestion
				Althea (15 per cent. extract)			
124	545	Atropin 4 mg. Althea 3 c.c. ...	0.0091 mg. 0.083 mg.	Increased respiration; moderate dyspnea	Killed, 47	Slight pulmonary distention, marked congestion, no hemorrhages; slight cardiac dilatation; slight abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion
160	400	Atropin 5 mg. Althea 3 c.c. ...	0.0125 mg. 1.25	Increased respiration	Killed, 38	Lungs collapsed, congested; punctate hemorrhages; no cardiac dilatation and no abdominal congestion	Moderate congestion, marked hemorrhage, marked edema, marked general distention
				Arsphenamin (0.5 per cent.)			
106	270	Atropin 6 mg. Arsphenamin 0.4 c.c.	0.022 mg. 0.008 mg.	Increased respiration; some restlessness	Killed,40	Lungs collapsed, slight congestion, not hemorrhagic; slight cardiac dilatation; moderate abdominal congestion	Moderate distention, marked congestion, hemorrhage, thrombosis

122	410	Atropin 4 mg. Aresphenamin 0.7 c.c.	0.01 mg. 0.0085 mg.	Increased respiration	Killed,31	Lungs distended, congested and hemorrhagic; moderate cardiac dilatation; slight abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion, hemorrhage, conglutination thrombi
				Typhobacterin (Mulford)			
114	400	Atropin 5 mg. Typhobacterin 2 c.c.	0.0125 mg. 0.005 mg.	Slight dyspnea, restlessness	Killed, 32	Moderate pulmonary distention and congestion, no hemorrhages; moderate cardiac dilatation; no abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion, slight hemorrhage, thrombosis
				Acacia (6 per cent.)			
116	530	Atropin 6 mg. Acacia 5 c.c. ...	0.0113 mg. 0.57 mg.	Some dyspnea; respiration increased	Killed, 30	Marked pulmonary distention, moderate congestion and marked hemorrhages; moderate cardiac dilatation and slight abdominal congestion	Slight distention, marked congestion, hemorrhage
120	680	Atropin 6 mg. Acacia 8 c.c. ...	0.0088 mg. 0.7 mg.	Increased respiration; moderate dyspnea, restlessness	Killed, 39	Slight pulmonary distention, marked congestion and few small hemorrhages; moderate cardiac dilatation and abdominal congestion; blood clots in 2 minutes	Slight distention, marked congestion
				Pollen extract (hay fever fall)			
113	440	Atropin 8 mg. Pollen extract 1 c.c. = 0.02 mg. nitrogen	0.018 mg. 0.0024 c.c.	Restlessness	Killed, 33	Very slight pulmonary distention, marked congestion and very minute hemorrhages; moderate cardiac distention and abdominal congestion; blood clotted in 3 minutes	Moderate distention, marked congestion, marked hemorrhage
121	470	Atropin 3 mg. (Hay fever pollen extract 1 c.c. spring) = 0.02 mg. nitrogen	0.007 mg. 0.0021 c.c.	Reslessness, jerky	Killed, 34	Pulmonary distention, moderate congestion; no hemorrhages; moderate cardiac dilatation; no abdominal congestion; blood clotted in 2½ minutes	Moderate distention, marked congestion, slight hemorrhage, conglutination thrombi

TABLE 2.—TREATMENT OF ANAPHYLACTOID PHENOMENA FROM VARIOUS AGENTS WITH EPINEPHRIN

Number	Weight of Guinea-Pig, Gm.	Total Dose Injected, C.c.	Dose per Gm. Guinea-Pig	Principal Symptoms	Fate of Animal Minutes	Necropsy	Microscopic Examination of Lungs
				Horse serum sensitization			
51	275	Epinephrin = 0.13 After serum = 0.5 c.c. to 3 c.c. N.S.	0.000048 mg. 0.00189 c.c.	Marked dyspnea convulsions; typical shock	Fatal, 3	Marked pulmonary inflation and congestion; pulmonary edema; heart slightly dilated; slight abdominal congestion; sensitized 20th day	Moderate distention, marked congestion, marked hemorrhage
57	220	Epinephrin = 0.1 together with serum = 0.5 c.c. to 3 c.c. N.S.	0.000046 mg. 0.0023 c.c.	Marked dyspnea; cyanotic twitching of ears; ruffling of hair; depression	Killed, 32	Lungs distended; minute hemorrhages; congestion slight, slight cardiac dilatation; marked abdominal congestion	Marked distention
100	270	Epinephrin = 0.013 (0.3 c.c. 1:10,000) 3 minutes before Serum 0.5 c.c.	0.00005 mg. 0.0018 c.c.	Convulsions; marked dyspnea; depression; death	Fatal, 5	Marked pulmonary inflation, hemorrhages and congestion; marked cardiac dilatation; no abdominal congestion; blood clotted in 5 minutes; 22d day of sensitization	Marked congestion, slight hemorrhage, conglutination thrombi
101	170	Epinephrin = 0.01 (0.1 c.c. 1:10,000) just before Serum 0.5 c.c.	0.00006 mg. 0.003 mg.	Marked dyspnea; restlessness; cyanosis; depression; gradual amelioration of symptoms	Killed, 30	Lungs moderately inflated, markedly congested and hemorrhagic; moderate abdominal congestion; blood clotted in 3 minutes; animal markedly emaciated; 22d day of sensitization	Marked congestion, marked hemorrhage
				Dextrin (6 per cent.)			
49	250	Epinephrin = 0.1 of 1:10,000 Dextrin 3 c.c......	0.00004 mg. 0.61 mg.	Marked dyspnea; depression; hair ruffled	Killed, 30	Lungs collapsed and markedly congested with many hemorrhages; cardiac dilatation and slight abdominal congestion	Marked congestion, hemorrhage, edema
				Agar sol (0.5 per cent.)			
56	205	Epinephrin = 0.1 of 1:10,000 3 c.c. 0.5 per cent. agar............	0.00005 mg. 0.073 mg.	Marked depression; dyspnea; cyanotic	Fatal, 7	Pulmonary distention and congestion; marked cardiac dilatation; moderate abdominal congestion	Moderate distention, marked congestion, hemorrhage, marked thrombosis, edema

				Agar sol gel (1 : 6)		
61	210	Epinephrin = 0.1 of 1:10,000 5 c.c. agar........	0.000048 mg. 0.02 mg.	Marked dyspnea increased respiration; irritable; air hunger	Killed, 30	Pulmonary distention; edema and congestion, also hemorrhages, distinct cardiac dilatation	Marked distention, marked congestion, marked hemorrhage
91	250	Epinephrin = 0.13 of 1:10,000 5 c.c. agar........	0.000052 mg. 0.017 mg.	Marked dyspnea; convulsions	Fatal, 8	Marked pulmonary distention, congestion and hemorrhages; slight cardiac dilatation, pericardial hemorrhages	Marked distention, marked congestion, marked hemorrhage
				Arsphenamin (0.5 per cent.)		
162	200	Epinephrin = 0.13 Arsphenamin = 0.6	0.00005 mg. 0.012 mg.	Respiration increased	Killed, 34	Lungs distended considerably, very hemorrhagic and moderately congested: no cardiac dilatation or abdominal congestion	Marked congestion, slight local distention
107	390	Epinephrin = 0.2 Arsphenamin = 0.68	0.000051 mg. 0.0087 mg.	Increased respiration; restlessness	Killed, 31	Lungs collapsed, markedly congested and hemorrhagic; moderate cardiac dilatation; very slight abdominal congestion	Slight distention, marked congestion, marked hemorrhage, thrombosis
				Peptone (10 per cent.)		
99	430	Epinephrin....... 0.1 c.c. peptone	0.00007 mg. 0.023 mg.	Markedly increased respiration only	Killed, 30	Pulmonary distention, numerous hemorrhages and congestion; cardiac dilatation and moderate abdominal congestion	Moderate distention, marked congestion, slight hemorrhage conglutination thrombi
157	550	Epinephrin....... Peptone = 0.033	0.00005 mg. 0.006 mg.	Markedly increased respiration	Killed, 50	Lungs inflated, markedly congested and hemorrhagic; marked cardiac dilatation and abdominal congestion	Marked congestion, marked hemorrhage, marked edema, conglutination thrombi, distention of unfilled alveoli
				Soluble starch (6 per cent.)		
161	300	Epinephrin = 0.15 Starch = 0.18	0.00005 mg. 0.6 mg.	Marked increase in respiration; hair ruffled	Killed, 36	Lungs moderately distended, markedly congested and hemorrhagic; cardiac dilatation; moderate abdominal congestion	Moderate congestion, slight hemorrhage, slight local distention, practically normal

TABLE 3.—SUMMARY OF PREVENTIVE POWER OF ATROPIN AND EPINEPHRIN AGAINST ANAPHYLACTOID PHENOMENA FROM VARIOUS AGENTS INJECTED INTRAVENOUSLY AS COMPARED WITH CONTROLS WITHOUT TREATMENT

Complete*	Partial	None
	Prevention by Atropin	
Beef serum, Whole	Agar sol, 0.5 per cent. (not fatal, otherwise no change)†	Acacia, 6 per cent. (same)
Peptone, 10 per cent.; small dose	Agar sol gel, 1:6 (fewer thrombi, otherwise same, doses larger; partial prevention to a certain extent)	Althea extract, 15 per cent. (about same)
	Arsphenamin, 0.5 per cent. (symptoms less severe, otherwise same; thrombi present)	Dextrin, 6 per cent. (worse, more distention)
	Beef serum, whole (not fatal)	Pollen extract, hay fever fall Mulford (about same, perhaps dyspnea less)
	Congo red, 1 per cent. (symptoms less severe; no distention)	Starch (about same)
	Peptone, 10 per cent.; larger dose (not fatal, otherwise same)	
	Serum-sensitization (not fatal; symptoms and necropsy changes less severe)	
	Typhobacterin, Mulford (symptoms less severe, otherwise same)	
	Prevention by Epinephrin	
	Arsphenamin, 0.5 per cent. (less dyspnea, otherwise same)	Agar sol, 0.5 per cent. (same; fatal)
	Serum-sensitization [not fatal, more pulmonary hemorrhage, otherwise same; epinephrin injected with antigen in one, and just preceding (less than 2 minutes) in the other animal]	Agar sol gel, 1:6 (same; fatal)
		Dextrin, 6 per cent. (same)
		Peptone, 10 per cent., small doses (worse thrombi and hemorrhages; otherwise same)
		Serum-sensitization (fatal; epinephrin injected after antigen in one and 3 minutes before in another animal)
		Starch, 6 per cent. (same)

* Means complete recovery as to absence of symptoms and necropsy, and microscopic changes in lungs, and approaching normal.

† Remarks in parenthesis refer to differences from controls injected with the same kind of agent and dose without atropin or epinephrin as the case may be.

to settle the question of how agar acts merely by experiments with intact animals. The matter can finally be settled by perfusion of the lungs. In anticipation of results along this line to be published later, it can be stated that agar acts differently from the various smooth muscle stimulants and, therefore, bears no relation to anaphylactic shock.

A word is necessary as to the mechanism of the partial amelioration obtained with atropin in the majority of our experiments. The following possibilities exist; (1) sufficient (moderate or even less) bronchial relaxation due to the atropin with prevention of the fatal effects of asphyxia; (2) improvement in or diminished circulatory disturbances such as cardiac dilatation, from paralysis of the parasympathetic endings by atropin; (3) respiratory stimulation by atropin with consequent amelioration of asphyxia; (4) ineffective central parasympathetic stimulation from asphyxia owing to paralysis of vagus endings by atropin. Whether any particular one or all of these factors are operative we do not know, and the conditions were not suitable for investigation of this.

However, it is quite patent that mere bronchial relaxation from atropin need not be assumed as responsible for the beneficial effects of relief that it may give, particularly with such a drug as arsphenamin, and probably also of congo red. If this is true, the further assumption that the arsphenamin reactions are a form of anaphylactic shock is certainly unjustified. This is believed to be the case. The results reported in our previous paper fortify this conclusion, since arsphenamin did not at all produce effects resembling those of anaphylaxis or anaphylactic shock. On the contrary, the disturbances were regarded to be of circulatory origin and, therefore, any amelioration that atropin may give is to be attributed to an improvement in the circulation rather than the supposed bronchiolar effects, at least in guinea-pigs. There is no good reason to believe that human individuals behave differently.

Atropin was found to give no demonstrable relief from the symptoms and disturbances produced by acacia, althea, dextrin, pollen extract and starch. Because of this and the reasons presented in our previous paper it is reasonable to conclude that the effects of these agents bear no relationship to anaphylaxis or anaphylactic shock.

Conclusions.—The intravenous injection of atropin in guinea-pigs in the dosage of 0.01 mgm. per gram of body weight can completely prevent the toxic effects produced by the intravenous injection of beef serum, and peptone in doses corresponding to those used in the proteose therapy of Nolf. Partial protection was obtained against the effects (principally symptoms of respiratory distress) of agar sol, agar sol-gel, arsphenamin, congo red, peptone (large doses), serum-sensitization (anaphylactic shock) and typhobacterin. No protection was obtained against the effects of acacia, althea, dextrin, pollen extract and starch.

2. *Prevention by Epinephrin*

As indicated by the summary in Table 3, protection with epinephrin against the anaphylactoid symptoms produced by various agents is even less than with atropin. Epinephrin itself (in the dosage used) produces rather profound effects such as increased respiration and pulmonary hemorrhages. Therefore, the experiments were limited to a number of the more important agents, namely, agar sol, agar sol-gel, dextrin, peptone, serum sensitization, starch and arsphenamin.

Partial protection was obtained only against two of these, namely arsphenamin and in serum-sensitization (anaphylactic shock). In the arsphenamin animals there was merely some amelioration of symptoms, the remaining effects being present as in the controls. This amelioration is probably mainly concerned with the circulation resulting in relief from the asphyxia due in turn to the depressant action of arsenic as in untreated animals. The effects of epinephrin on previously untreated (unconstricted) bronchioles being indeed doubtful, and this taken together with the possibilities indicated above with atropin, and the fact that the effects of arsphenamin in control animals do not resemble those of true anaphylactic shock, confirms the conviction that whatever beneficial effects epinephrin may have in the disturbances from arsphenamin are concerned with improvement in the circulation. The burden of proof rests on those who attribute the benefits to bronchiolar relaxation, and consequently amelioration of anaphylactic shock or anaphylaxis.

It is seen that the protective powers of epinephrin in true anaphylactic shock vary with the time of administration of the drug (see Tables 2 and 3). In two animals death was prevented when the epinephrin was injected together with and just preceding the injection of the antigen (horse-serum). On the other hand, when a greater interval elapsed between the injection of epinephrin and antigen, the two animals injected died of anaphylactic shock in the usual way. In one animal the epinephrin was injected three minutes before and in the other some time after the antigen when the conditions for eliciting the symptoms of anaphylactic shock were under mobilization and leading to a fatal issue from the bronchiolar spasm. These results agree perfectly with the well known pharmacologic actions of epinephrin on the bronchi. That is, epinephrin is effective as a dilator only on constricted bronchioles, when it is present in effective concentration, which must be at the time constriction is occurring or just preceding it. After the bronchiolar constriction has occurred, as it does rather violently and with remarkable rapidity in anaphylactic shock, epinephrin is no longer active because a fatal issue has practically supervened. Larger doses might perhaps still be effective, but these were practically precluded in our experiments with guinea-pigs because of the extensive pulmonary hemorrhages produced by epinephrin itself and the consequent complications. As indicated by the results obtained, epinephrin is effective as a prophylactic in anaphylactic shock of guinea-pigs when injected together with the antigen or immediately preceding it.

These results with epinephrin in anaphylactic shock throw some light on the mechanism of agar action and similar colloids. We have seen how epinephrin acts as a prophylactic in anaphylactic shock when injected under proper conditions, and this is due, no doubt, to an alleviation of the bronchoconstriction as indicated by the diminished pulmonary distention at necropsy, and other effects. Epinephrin was injected in the same way in the experiments with agar, but practically no protection whatsoever was obtained. In fact, certain effects (pulmonary hemorrhages) were accentuated. The fatalities were greater. This is so because the mechanism through which epinephrin exerts its beneficial effects is absent in agar action, namely, active bronchoconstriction. Therefore, the pulmonary distention from agar and similarly acting agents cannot be due to the same cause as in anaphylaxis or anaphylactic shock.

Besides being inefficient against agar, epinephrin furnished no protection against the effects of dextrin, and small doses of peptone and starch. On the contrary, the incidence of pulmonary hemorrhages was increased. experimentally at least, the protective power of epinephrin in the proteose therapy of Nolf has not been confirmed.

Conclusions.—When injected together with the antigen or immediately preceding it, epinephrin in the dosage of 0.0005 c.c. of 1 : 10,000 per gram of body weight intravenously prevents death from true anaphylactic shock in guinea-pigs. The partial protection afforded by epinephrin intravenously against the symptoms from arsphenamin disturbances is attributed to circulatory improvement. Epinephrin exerts no protection against the effects of agar sol, agar sol-gel, dextrin, peptone (small doses), starch, and when injected after or too long before the antigen in serum sensitized animals.

On the basis of the results obtained with atropin and epinephrin, the mechanism of action of agar and similar agents bears no relationship to true anaphylaxis or anaphylactic shock.

IV. CONCLUSIONS

1. The intravenous injection of atropin in guinea-pigs in the dosage of 0.01 mgm. per gram of body weight can completely prevent the toxic effects produced by the intravenous injection of beef serum; and peptone in doses corresponding to those used in the proteose therapy of Nolf. Partial protection was obtained against the effects (principally symptoms of respiratory distress) of agar sol, agar sol-gel, arsphenamin, congo red, peptone (larger doses), serum-sensitization (death in anaphylactic shock) and typhobacterin. No protection was obtained against the effects of acacia, althea, dextrin, pollen extract and starch.

2. When injected together with the antigen or immediately preceding it, epinephrin in the dosage of 0.0005 c.c. of 1 : 10,000 per gram of body weight intravenously prevents death from true anaphylactic shock in quinea-pigs. The partial protection afforded by epinephrin (intravenously) against the symptoms of arsphenamin disturbances is attributed to circulatory improvement. Epinephrin exerts no protection against the effects of agar sol, agar sol-gel, dextrin, peptone (small doses), starch and when injected after or too long before the antigen in serum-sensitized animals.

On the basis of the results obtained with atropin and epinephrin, the mechanism of action of agar and similar agents bears no relationship to true anaphylaxis or anaphylactic shock.

EFFECTS OF VARIOUS COLLOIDS AND OTHER AGENTS WHICH PRODUCE ANAPHYLACTOID PHENOMENA ON BRONCHI OF PERFUSED LUNGS *

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I. INTRODUCTION

The results previously reported [1] with agar and similar colloids on untreated guinea-pigs and those treated with atropin and epinephrin [2] indicated no relationship between the pulmonary changes and effects that were produced and true anaphylaxis or anaphylactic shock. However, the pulmonary distention produced by agar is so marked, even after prophylactic treatment with atropin and epinephrin, that further proof is required in order to disprove definitely the analogy between this and anaphylactic shock. Moreover, objection might be raised against the too free interpretation of our results in intact animals. So far as bronchiolar effects are concerned this can be settled by perfusion of the surviving lungs. It is the object, therefore, of this paper to report the results with agar and other agents on bronchioles directly in surviving lungs. Arsphenamin was also tried in order to make the proof still more conclusive against this agent.

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[1] Hanzlik and Karsner: J. Pharm. Exp. Therap. **14**: 379, 1920.

[2] Hanzlik and Karsner: J. Pharm. Exp. Therap. **14**: 425, 1920.

II. METHOD

The lungs of guinea-pigs were perfused according to the method of Baehr and Pick.[3] All animals were previously rather deeply anesthetized with ether. A glass T-tube was tied into the trachea and one limb of this was joined to an artificial respiration apparatus registering about 30 strokes per minute. The chest was split open; the pericardium slit, the pulmonary artery freed and two silk ligatures carefully placed underneath it with a small aneurysm needle. Traction with the ligature toward the operator was made so as to shut off the blood from the right ventricle momentarily, and at the same time a long glass cannula was tied in, pointing toward the lungs. The cannula was then quickly connected with normal saline (in Mariotte bottles suspended on a small perfusion rack) and the left ventricle was quickly transected to allow escape of the perfusion fluid. The artificial respiration which had been applied in the meantime was continued throughout the experiment, regulating the escape of air from the tracheal T-tube so as to permit complete collapse of the lungs during the expiratory phase.

Complete permanent distention of the lungs in the inspiratory phase means bronchoconstriction as from histamin, peptone or serum with lungs of sensitized animals. However, care must be exercised in interpreting the failure to collapse from edema. When edema occurs the perfusion fluid as a rule begins to issue from the trachea and the lungs appear soggy and very wet.

If the operation has been successful, the blood is washed out of the lungs within a minute or two and the lungs continue to distend and collapse for an hour or more when edema occurs and the preparation must be discarded. On the other hand, if the blood is incompletely washed out, which always occurs if there is too much delay in the operation after the pulmonary artery has been incised, then clots remain in the pulmonary vessels and capillaries and the result is a very prompt and marked distention simulating the action of histamin, peptone and serum-sensitized lungs. In such an event, the blood and clots are easily recognizable, which is not the case when the operation is entirely successful and the inflation occurs. A properly perfused lung which has become at the same time distended from an active bronchoconstrictor like histamin or peptone is fluffy, very pale and floats on ether. We always took special care to note the presence or absence of blood in our preparations microscopically, since this was of very great importance in properly interpreting the results with agar, which forms emboli and thrombi, the effect of which is the same as of blood clots, fibrin, etc. This will be referred to again later.

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[3] Baehr and Pick: Arch. f. exp. Path. Pharm. **74**: 65, 1913.

In our experiments ordinary saline (0.9 per cent. NaCl) was used as the vehicle for the drugs, although Tyrode's or Ringer's solution is better for perfusion experiments. Most of the animals used weighed about 400 grams each.

The concentrations of the different agents corresponded to those occurring in the blood of intact animals as used in the experiments previously reported (1, 2) by us. In several instances, notably with acacia and arsphenamin, the concentrations corresponded to those occurring in human blood after therapeutic doses of these agents. The different agents were dissolved in normal saline and prepared in exactly the same way as used in our former experiments with intact animals. All the solutions were filtered through a quantitative filter paper (Whatman No. 40) before they were used for perfusion. For details of preparation, etc., the first paper[1] of this series should be consulted.

Certain of the lungs perfused with agar, acacia and peptone were also treated with atropin and papaverin to help ascertain if the distention which occurred was due to bronchoconstriction or not. The peptone constriction is known to be relieved by atropin and served as a control.

After the perfusion was completed, small portions of the lungs were placed at once into Zenker's fluid, later stained and sectioned in the usual way and examined miscroscopically for the presence of blood, emboli, distention, etc. The importance of this was pointed out above. So far as distention is concerned, the presence of moderate or slight local distention is of no significance, since the lungs in these preparations are purposely rather strongly distended by artificial respiration and a moderate amount of air is apt to be retained even in collapsed lungs, particularly when edema is present. Therefore, gross pulmonary distention is of far greater significance.

III. DISCUSSION OF RESULTS

1. Agar and Active Bronchoconstrictors

The results with these agents are presented in Table 1. Peptone and histamin and horse serum (in sensitized lung) produced marked distention. These agents are known to stimulate bronchial muscle directly and served as controls for the results with agar sol-gel (1 :6). Of these histamin was most active, producing a marked pulmonary distention in forty-five seconds, while peptone and serum in sensitized lung required about four and two minutes, respectively. Microscopically, no emboli or blood were present with such active bronchoconstrictors as peptone and in the serum sensitized lung.

On the other hand, the lungs perfused with agar sol-gel invariably showed the presence of agar emboli and there was a prompt stoppage of the perfusion flow through the lungs. The emboli were composed of clumps of agar in the pulmonary vessels and capillaries. In other respects the results with agar perfusion were precisely the same as with peptone and the serum sensitized lung, namely, a very marked, prompt and pale gross distention of the lungs occuring in two to three minutes after the perfusion flow was started. The lungs were lighter than ether, indicating presence of distention and absence of edema. Experiments 129b and 149b represent perfusions of one lung each of two preparations previously perfused with dextrin and acacia, respectively. The lungs continued to collapse with dextrin and acacia, but when agar sol-gel was perfused through the unligated lung of each preparation they promptly distended in the same way as previously untreated lungs, indicating conclusively that dextrin and acacia do not inflate, confirming other experiments with these agents to be described presently, and that agar does so rather peculiarly. Unfortunately, the particular microscopic sections reveal no agar emboli or thrombi. However, the perfusion flow stopped promptly and there is every reason to believe that emboli would have been found if sections had been more fortunately selected. Greater significance is attached to the presence of emboli in untreated lungs than in these two lungs previously perfused with dextrin and acacia. Moreover, in experiments 70, 126 and 147 sections from both lungs were made and, therefore, the emboli were more adequately revealed.

TABLE 1.—PERFUSION OF LUNGS WITH AGAR, PEPTONE, HISTAMIN AND HORSE SERUM

Number of Experiment	Complete and Permanent Gross Pulmonary Distention in (Minutes)	Microscopic Examination of Perfused Lung †
	Agar sol gel (1: 6)	
70	3	Agar Emboli; marked distention
126	3	Masses of agar in large vessels; moderate distention
129b	3	Moderate local distention; alveoli normal
147	2	Many agar emboli; marked distention
149b	2	Local distention; alveoli normal
	Peptone	
136	3½ (0.03% peptone)	Marked distention only
D*	4 (1% peptone)	Marked distention only
	Horse serum (2.8 per cent.; lung of sensitized animal)	
135	2	Moderate local distention; alveoli normal (gross distention very marked)
	Histamin (1: 100,000)	
D*	¾	(Gross distention very marked)

* Class demonstration experiment.

† All of the lungs in this table floated on ether and edema was absent.

These results indicate definitely that the mechanism of agar inflation is peripheral, local and probably mechanical owing to the presence of emboli. However, the results thus far do not as yet constitute absolute conclusive proof against plain muscle stimulation as a factor coöperating with the emboli in reducing the caliber of the bronchi. That is, without the microscopic evidences of agar emboli, it would be impossible to differentiate the results of the experiments with agar from those with peptone and histamin, and the interpretation would be pulmonary distention from bronchoconstriction due to direct stimulation of bronchial muscle or the parasympathetic endings. This can be definitely resolved by means of atropin and papaverin.

2. *Effects of Atropin and Papaverin*

Tonically constricted bronchial musculature as from peptone, in sensitized lungs perfused with serum (anaphylactic), etc., can be relaxed when the lung preparation is perfused with atropin. Even histamin, perhaps the most powerful bronchoconstrictor known, can be antagonized by atropin (Baehr and Pick[3]), although this is difficult with low concentrations. In our experiments, high concentrations of atropin, namely 0.05 per cent. and 0.1 per cent., were used.

The treatment with atropin was carried out in two ways: (1) in one set of experiments the lungs were perfused after inflation occurred, and (2) in another set of experiments preliminary perfusion of the lungs was carried out. Agar, acacia and peptone were studied in this way with atropin and agar with papaverin. The results are presented in Table 2.

These indicate that atropin has no effect on the pulmonary distention produced by agar sol-gel (1 : 6). Distention occurred promptly, that is, in three minutes, and the pulmonary vessels were loaded with agar emboli in the absence of blood in the same way as unatropinized lungs. On the other hand, the effects from peptone, which is a parasympathetic stimulant, were antagonized by atropin. This shows that the inflation by agar is not due to bronchoconstriction from stimulation of parasympathetic endings.

TABLE 2.—EFFECT OF ATROPIN AND PAPAVERIN ON LUNGS PERFUSED WITH AGAR, ACACIA AND PEPTONE

Number of Experiment	Agent	Changes in Perfused Lung	Microscopic Examination of Perfused Lung and Remarks
Atropin sulphate (0.1 per cent.) after perfusion with agent			
126	Agar sol gel (1: 6)	Distended in 3 minutes; no collapsing in 5 minutes after atropin	Masses of agar in large vessels; moderate distention; lungs floated on ether
128	Acacia (2%)	Partial distention in 9 minutes; no collapsing in 9 minutes after atropin	Fibrin thrombi in large vessels; lungs floated on ether
136	Peptone (0.03%)	Distention in 4 minutes; some collapsing in 10 minutes after atropin	Marked distention; lungs floated on ether
Preliminary perfusion with atropin sulphate			
144	Agar sol gel (1: 6)	Atropin (0.1 per cent.) perfused for 4 minutes; complete distention in 3 minutes after agar was started	Agar emboli in large vessels; moderate distention; lungs floated on ether
148	Agar sol gel (1: 6)	Atropin (0.1 per cent.) perfused for 5 minutes; complete marked distention in 2 minutes after agar was started	Many agar emboli; moderate distention; lungs floated on ether
145	Acacia (1.7%)	Atropin (0.1 per cent.) perfused for 5 minutes; collapsed well for 20 minutes after acacia was started	Slight local distention; lungs submerged in ether
150	Peptone (0.05%)	Atropin (0.05 per cent.) perfused for 5 minutes before peptone was started; collapsed well for 20 minutes	Slight local distention; alveoli normal; lungs sank in ether
Papaverin HCl (0.1 per cent.)			
163	Agar sol gel (1: 6)	Papaverin perfused for 5 minutes; marked distention in 3 minutes after agar was started; perfusion flow stopped	Lungs floated on ether; moderate local distention; agar thrombi in large vessels; no bronchoconstriction
164	Agar sol gel (1: 6)	Papaverin perfused for 6 minutes; partial distention in 6 minutes, complete and marked distention in 9 minutes after agar was started	Lungs floated on ether; moderate local distention; agar thrombi in larger vessels; no bronchoconstriction
165	Agar sol gel (1: 6)	Papaverin perfused for 4 minutes before, then papaverin together with agar for 5 minutes, when perfusion flow stopped and lungs were markedly and completely distended	Lungs floated on ether; marked distention (local in one section, general in the other); no thrombi

The results with papaverin were also negative, indicating definitely that the inflation from agar is not due to stimulation of bronchial muscle itself. That agar is not a smooth muscle stimulant will be further supported by the negative results obtained with surviving intestine and uterus to be described in a subsequent paper.

These results prove conclusively that inflation of lungs by agar is entirely peripheral, local and mechanical in the absence of active bronchial stimulation. This is also the main mechanism in the agar inflation of intact animals. The greatest significance is attached to the presence of agar emboli in perfused lungs, and thrombi of the pulmonary vessels in intact animals. Bearing in mind this and the results of our analysis the mechanism of agar action appears to be as follows.

Mechanism of Agar Action.—The agar forms thrombi, which block the small pulmonary vessels, surrounding the bronchioles, which in turn are compressed by the *vis a tergo* of the pulmonary flow from the right heart of the intact animal, and of the perfusion fluid in surviving lungs. In the perfused lung the flow of perfusion fluid stops. The compressed bronchioles are sufficiently narrowed in caliber to prevent the escape of the air forced in by the violent efforts of inspiratory dyspnea of the intact animal and the artificial respiration of the perfused lungs. In other words, there is a passive bronchoconstriction (really a compression of the bronchi), and as a result permanent inflation of the lung. Since there is no increased excitability of parasympathetic endings or bronchial muscle, it is obvious why atropin and papaverin are ineffective in perfused lungs, and atropin and epinephrin in intact animals. That the effect is purely peripheral, local and mechanical is attested to by the results with lung perfusion, and, on this account the conclusion is forced that this is the principal and primary mechanism of agar action in intact animals. Although, of course, as a result of the asphyxia which supervenes after the pulmonary inflation develops and respiratory gaseous interchange is interfered with, it is conceivable that additional bronchoconstriction is superimposed as a result of central asphyxial stimulation of the vagus (parasympathetic) on the passive constriction primarily brought about. Asphyxial stimulation is probably the principal mechanism with other agents (colloids and others) that do not effectively occlude the pulmonary vessels of intact and perfused lungs, and in which circulatory disturbances play the leading rôle. However, with agar, we believe that passive bronchoconstriction by compression from emboli and thrombi as explained above is the primary determining mechanism of the pulmonary distention and subsequent phenomena.

3. *Inactive Agents*

The results presented in Table 3 indicate that the following agents: acacia, althea, arsphenamin, congo red, dextrin, gelatin, glycogen, nuclein solution and soluble starch, do not distend perfused surviving lungs. Therefore, they do not cause bronchoconstriction peripherally. The pulmonary inflations which occurred in intact animals, even though relatively moderate and inconstant, with the following: acacia, dextrin, althea, starch, congo red and gelatin, might be of central origin as a result of circulatory injury, which accompanies the effects, and due to asphyxia. So far as perfused lungs are concerned, emboli were not demonstrable with althea, congo red, dextrin, gelatin, glycogen, nuclein solution and starch, and thrombi were also absent in intact animals.

Acacia produced distention in two out of five lungs that were perfused although this was not prompt and one of the lungs was found to contain fibrin (blood) thrombi on microscopic examination which is sufficient to account for the inflation. Bronchoconstriction was finally excluded by means of atropin (see Table 2) The majority of animals injected with acacia intravenously showed the presence of pulmonary thrombi, but the perfused lungs were uniformly negative as to this. Therefore, their occurrence in the circulation of the intact animal must be of greater significance. There

TABLE 3.—PERFUSION OF LUNGS WITH VARIOUS COLLOIDS AND ARSPHENAMIN

Number of Experiment	Gross Pulmonary Distention Absent End of Minutes	Microscopic Examination of Perfused Lungs	Remarks
		Acacia (1.7 per cent.)	
127	10		Lungs floated on ether
127	20*	Marked distention............	Lungs floated on ether; edematous
128	9†	Marked distention............	Fibrin thrombi in few veins; 2 per cent. acacia used Lungs floated on ether; edema present
146	15	Distended in some areas only	Lungs sink in ether; edema marked
149a	14	Moderate local distention; alveoli normal	Lungs floated on ether
		Althea (2.5 per cent.)	
130	9	Marked distention............	Lungs sank in ether; very edematous
131	20	Moderate local distention; alveoli normal	Edema present
		Arsphenamin (0.01 and 0.02 per cent.)	
139	17	Marked distention in local areas; alveoli normal; golden brown masses size of erythrocytes and others smaller, resembling hemosiderin granules	0.01 per cent. arsphenamin used; lungs floated on ether
140	15	Irregular distention; no pigment	0.02 per cent. arsphenamin used; marked edema present
		Congo red (0.17 per cent.)	
132	15	Slight local distention; alveoli normal; conglutination thrombi in numerous capillaries; corpuscles stained orange; no blood in larger vessels	Lungs sank in ether; marked edema
133	18	Same as in Experiment 132.	Marked edema present
		Dextrin (1 per cent.)	
129a	22	Moderate local distention; alveoli normal	Lungs floated on ether
138	18	Marked distention; alveoli normal	Lungs hemorrhagic; float on ether
		Gelatin (1 per cent.)	
134a	16	Marked distention............	Lungs floated on ether; edema present
		Glycogen (0.1 per cent.)	
137	16	Marked distention; alveoli normal	Actual concentration of glycogen = 0.083 per cent., lungs sank in ether; edema present
163	15	Marked distention............	Edema present
		Nuclein solution (0.1 per cent.)	
143	20	Marked distention............	Lungs submerge in ether; marked edema
		Soluble starch (1 per cent.)	
141	15	Few areas of local distention; alveoli normal	Lungs sink in ether; some edema
142	16	Areas of distention more numerous; alveoli normal	Lung floats on ether; edema present

is no reason to doubt that these would not act like agar thrombi and produce a passive bronchoconstriction, although the effects may well be mixed with asphyxia from circulatory injury arising primarily or together with the blocking of the pulmonary vessels. In another paper we shall submit evidence that acacia agglutinates blood corpuscles *in vitro.* This would tend to support the mechanism of thrombi formation *in vivo* with its consequent deleterious effects.

Concerning arsphenamin, it can be said that this was entirely negative as to lung inflation, and, therefore, peripheral bronchoconstriction is excluded. It was previously shown that pulmonary inflation was also absent in intact animals. All of these results taken together prove conclusively that the disturbances from the intravenous injection of arsphenamin bear no relationship whatsoever to anaphylactic shock or anaphylaxis.

IV. CONCLUSIONS

1. Perfusion of the lungs of guinea-pigs with agar sol-gel (1 : 6) causes prompt and marked distention of the lungs and stoppage of the perfusion flow due to the massing of agar emboli in the pulmonary vessels.

2. Prompt distention of perfused lungs occurs with peptone and histamin, but in the absence of pulmonary emboli, and this is due to active stimulation of bronchial muscle.

3. The distention from agar is not due to direct stimulation of bronchial musculature or parasympathetic endings, since the effects are not antagonized by papaverin and atropin.

4. The mechanism of agar action consists of a passive bronchoconstriction by compression of bronchioles from emboli in the pulmonary vessels.

5. Passive bronchoconstriction is also the chief mechanism in the marked pulmonary inflation produced by agar in intact guinea-pigs, whose pulmonary vessels contain thrombi invariably, although, of course, a superimposed bronchial stimulation from central stimulation occurring as a result of the supervening asphyxia is conceivable as a secondary phenomenon.

6. The following agents produced no gross distention of perfused lungs, therefore, no peripheral bronchoconstriction: acacia, althea, arphenamin, congo red, dextrin, gelatin, glycogen, nuclein solution and soluble starch. Whatever inflation these agents produce (though variably) in intact animals, appears to be of central origin due to asphyxia arising from circulatory injury, which exists. When the inflation is absent the effects are entirely circulatory (arsphenamin).

EFFECTS OF VARIOUS COLLOIDS AND OTHER AGENTS WHICH PRODUCE ANAPHYLACTOID PHENOMENA ON SURVIVING INTESTINE AND UTERUS *

PAUL J. HANZLIK

CLEVELAND

(From The Journal of Pharmacology and Experimental Therapeutics, February, 1920, p. 463)

OBJECT

All the evidences that have been thus far obtained by us [1] indicate that the marked inflation of the intact and surviving lungs, produced by agar, is not due to direct stimulation of bronchial muscle or parasympathetic endings. The effects of various other nonprotein colloids are variable, though quite similar with some, notably acacia. The mechanism of action of these agents was discussed in a previous communication. However, in order to make the proof against smooth muscle stimulation by these agents still more conclusive, experiments have been made on surviving intestine and uterus.

METHODS

The surviving intestine (longitudinal and circular strips) of rabbits and guinea-pigs and longitudinal strips of rabbit's virgin uterus were used. The strips were conveniently attached to weighted levers, which recorded the movements of the organs on a slow moving kymograph. For immersion, 55 c.c. of Ringer's solution (mammalian) was used in tall glass cylinders resting in a large water bath maintained at 38 C. The various agents were added after peristalsis was fairly constant.

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* From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland.

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[1] Hanzlik and Karsner: J. Pharm. Exp. Therap. **14**: 379, 425, 449, 1920.

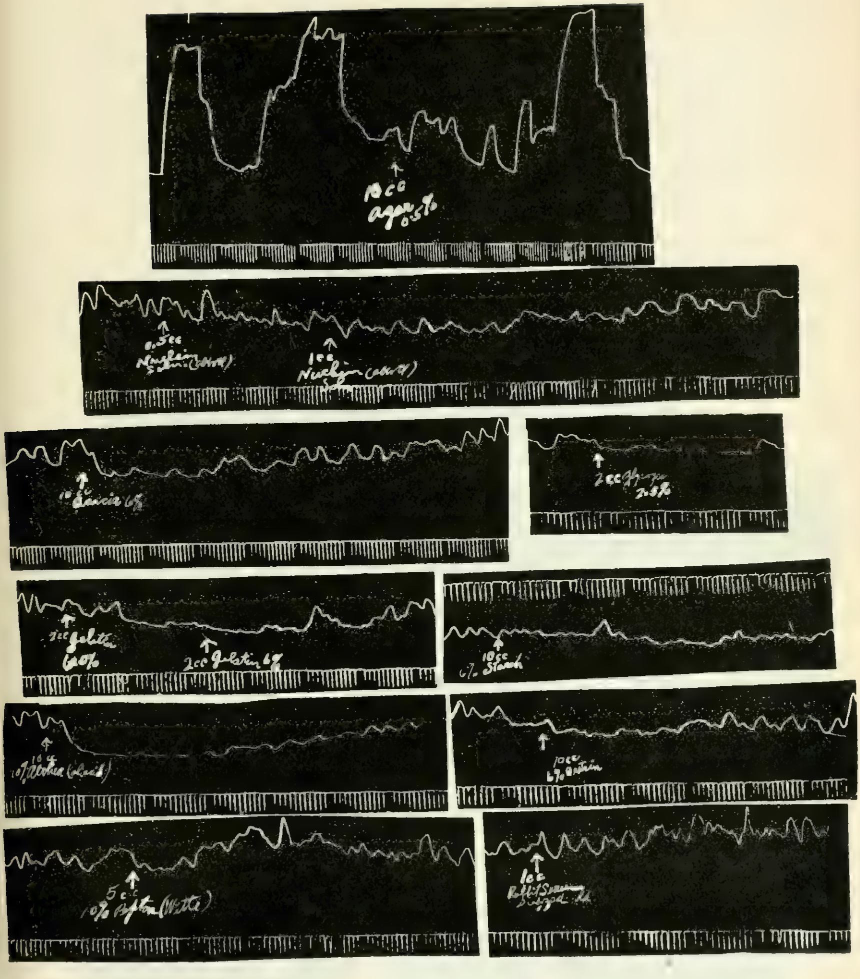

Fig. 1 (Experiment 167).—Showing effects of agar (1 per cent.), acacia (1.7 per cent.), Starch (1.2 per cent.), glycogen (0.1 per cent.), althea (2 per cent.), dextrin (1.2 per cent.), gelatin (1.2 per cent.), nuclein solution (1 c.c. = 0.02 per cent.), peptone (1 per cent.), and rabbit's serum (0.2 per cent.) on surviving rabbit's uterus in Ringer's solution (50 c.c.). The figures in parentheses denote end concentrations. Time: each stroke = 5 seconds. Depression was produced by all agents except peptone and rabbit's serum which acted as controls and stimulated uterine persistalsis.

The concentrations of the different agents varied. These ranged from concentrations occurring in blood after the intravenous injection of these in the experiments on systemic actions previously reported [1] up to very high concentrations in order to see if augmentor effects could be obtained.

None of the animals had been previously treated with any of the agents used. The experiments on rabbit's uterus agreed so closely with those on the intestine of both rabbit and guinea-pig that it was deemed unnecessary to carry out additional experiments with guinea-pig's uterus.

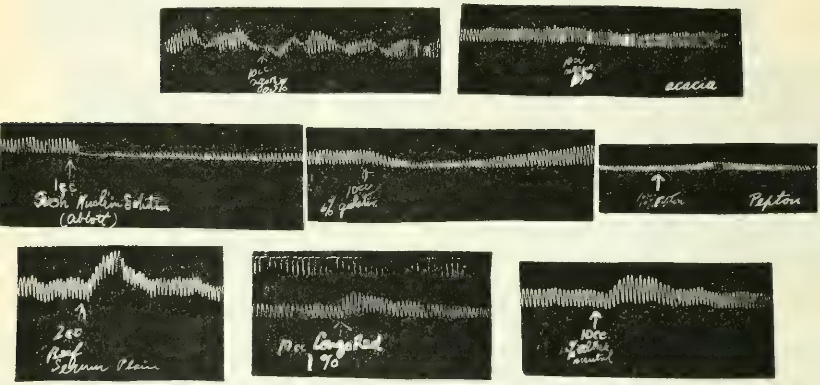

Fig. 2 (Experiment 166).—Showing effects of agar (1 per cent.), acacia (2 per cent.), nuclein solution (0.02 per cent.), congo red (0.2 per cent.), peptone (0.2 per cent.), beef serum (4 per cent.), and neutral althea (2 per cent.) on longitudinal strip of rabbit's intestine in Ringer's solution (50 c.c.). The figures in parentheses denote end concentrations. Depression was produced by agar, acacia, nuclein solution and gelatin; stimulation by peptone, beef serum, congo red and neutral althea.

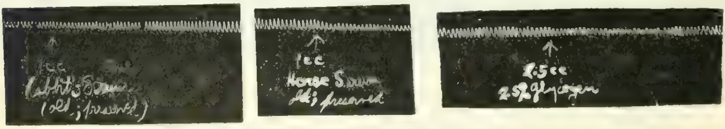

Fig. 3 (Experiment 165).—Showing effects of rabbit's serum (0.2 per cent.), horse serum (0.2 per cent.), and glycogen (0.1 per cent.) on longitudinal strip of rabbit's intestine in Ringer's solution (50 c.c.). The figures in parentheses denote end concentrations of the different agents. Depression was produced by horse serum and glycogen; stimulation by rabbit's serum.

RESULTS

The results with surviving intestine are presented in Table 1, and those with uterus in Table 2. These indicate also the various concentrations that were used and conditions under which the experiments were performed. In addition, three figures, illustrating typical effects of the more important agents on the intestine and uterus, are included.

The results (as indicated by the data in the tables, and the figures) are so uniform and striking that further interpretation is practically unnecessary.

SUMMARY

The main results may be stated as follows:

Agar produces no effects on or slight depression of the peristalsis of surviving intestine and uterus. The following agents uniformly depressed both the intestine and uterus: acacia, dextrin, glycogen, gelatin, starch, human and horse serums and nuclein solution (Abbott). Althea extract was variable, although depression was the rule, no matter whether slightly acid, alkaline or neutral in reaction. The following agents produced moderate to marked stimulation of both uterine and intestinal peristalsis: peptone, congo red and rabbit's serum. Beef serum was irregular. So far as peptone and rabbit's serum are concerned the results obtained are confirmative of the usual effects, and these agents served as controls for the inactive agents, indicating that the organs were functionally active and responsive in the usual way when tested against well known augmentors of peristalsis. The results with congo red do not agree with those on surviving lungs in which inflation (bronchoconstriction) did not occur. However, this is immaterial to the results of this investigation.

So far as agar, acacia and the remaining nonprotein colloids are concerned, the results confirm those obtained on surviving lungs. That is, these agents are not stimulants of plain muscle in the bronchi, uterus and intestine. On the contrary, they almost invariably depressed the intestinal and uterine muscle. This

TABLE 1.—EFFECTS OF PEPTONE, SERUMS AND VARIOUS NONPROTEIN COLLOIDS ON SURVIVING INTESTINE

No. of Experiment.	Agent	End Concentration in Ringer's Solution, per Cent.	Species	Effect on Peristalsis	Kind of Strip
			Peptone (10 per cent.)		
165	Peptone	0.01	Rabbit	Slight stimulation; increased amplitude	Circular
	Peptone	0.03	Rabbit	Slight stimulation; increased amplitude	Circular
	Peptone	0.2	Rabbit	Marked stimulation; increased amplitude	Circular
	Peptone	1.0	Rabbit	Moderate stimulation; increased amplitude	Circular
165	Peptone	0.03	Rabbit	None	Longitudinal
	After acacia	0.2			
	After acacia	2.0	Rabbit	None	Longitudinal
	Peptone	0.2	Rabbit	Moderate stimulation	Longitudinal
	After acacia	0.2			
165	Peptone	0.2	Rabbit	Gradual stimulation, increased amplitude	Longitudinal
	After rabbit's serum	2.0			
166	Peptone	0.2	Rabbit	Mild stimulation	Longitudinal
	After agar	0.05			
	Peptone	0.03	Rabbit	None	Longitudinal
	After agar	0.05	Rabbit		
	Peptone	0.03	Rabbit	None	Longitudinal
	After dextrin	1.2			
			Acacia (6 per cent.)		
165	Acacia	0.1	Rabbit	Depression with prompt recovery; lessened amplitude	Longitudinal
165	Acacia	1.0	Rabbit	Marked depression of amplitude; gradual recovery	Longitudinal
166	Acacia	1.0	Rabbit	Moderate depression of amplitude; partial recovery	Longitudinal
166	Acacia	1.0	Rabbit	Abolition of peristalsis, complete recovery on removal of acacia	Longitudinal
165	Acacia	1.0		Marked depression of amplitude; gradual recovery	Longitudinal
	After Congo red	0.02	Rabbit		
			Dextrin (6 per cent.)		
165	Dextrin	0.6	Rabbit	Moderate depression; lessened amplitude and rate	Longitudinal
165	Dextrin	0.6	Rabbit	Gradual abolition of peristalsis, slow recovery	Longitudinal
166	Dextrin	1.2	Rabbit	Moderate depression of amplitude; no recovery	Longitudinal
			Glycogen (2.5 per cent.)		
165	Glycogen	0.1	Rabbit	Practically no effect	Longitudinal
165	Glycogen	0.1	Rabbit	None	Longitudinal

166	Glycogen	0.1	Rabbit	None	Longitudinal
166	Glycogen	0.1	Rabbit	None	Longitudinal
			Gelatin (6 per cent.)		
165	Gelatin	1.2	Rabbit	Marked depression in rate and amplitude, slow, gradual recovery	Longitudinal
166	Gelatin	1.2	Rabbit	Moderate depression; rapid recovery	Longitudinal
166	Gelatin	1.2	Rabbit	Marked prompt depression of rate and amplitude; gradual recovery	Longitudinal
166	Gelatin	1.2	Rabbit	Abolition of peristalsis	Longitudinal
			Soluble Starch (6 per cent.)		
165	Starch	1.2	Rabbit	Moderate stimulation; increase in amplitude gradual	Longitudinal
166	Starch	1.2	Rabbit	Momentary insignificant depression of amplitude	Longitudinal
			Serums (whole)		
165	Human (old; preserved with 0.3 per cent. trikresol)	0.4	Rabbit	Mild transitory depression of amplitude	Longitudinal
165	Human (old; preserved with 0.3 per cent. trikresol)	2.0	Rabbit	More prolonged depression of amplitude	Longitudinal
165	Human (old; preserved with 0.3 per cent. trikresol)	2.0	Rabbit	More prolonged depression of amplitude	Longitudinal
166	Human (old; preserved with 0.3 per cent. trikresol)	4.0	Rabbit	Abolished peristalsis for short period, gradual recovery	Longitudinal
165	Rabbit serum (old); undecomposed	2.0	Rabbit	Moderate stimulation; increased amplitude	Longitudinal
165	Rabbit serum (old); undecomposed	2.0	Rabbit	Stimulation; increased amplitude	Longitudinal
165	Rabbit serum (fresh)	4.0	Rabbit	Stimulation moderate	Longitudinal
166	Rabbit serum (old; preserved with trikresol)	4.0	Rabbit	Almost total abolition of peristalsis, recovery on removal of serum	Longitudinal
165	Horse serum (old; preserved with trikresol)	2.0	Rabbit	Depression of peristalsis (decreased amplitude)	Longitudinal
165	Beef serum (old; preserved with trikresol)	2.0	Rabbit	Abolition of peristalsis	Longitudinal
166	Beef serum (plain; old) undecomposed	2.0	Rabbit	Mild stimulation; increased tone and rate	Longitudinal
166	Beef serum (plain; old) undecomposed	4.0	Rabbit	Marked stimulation; increased tone rate and amplitude somewhat	Longitudinal
			Congo Red (1 per cent.)		
165	Congo red	0.2	Rabbit	Marked stimulation; increased tone and amplitude	Longitudinal
165	Congo red	0.02	Rabbit	Moderate lasting stimulation	Longitudinal
165	Congo red	0.02	Rabbit	Considerable stimulation (chiefly increased amplitude)	Longitudinal
166	Congo red	0.08	Rabbit	Marked stitmulation (increased tone and amplitude)	Longitudinal
166	Congo red	0.2	Rabbit	Definite stimulation (increased amplitude)	Longitudinal
166	Congo red	0.02	Rabbit	Moderate stimulation (increased amplitude)	Longitudinal

TABLE 1—EFFECTS OF PEPTONE, SERUMS AND VARIOUS NONPROTEIN COLLOIDS ON SURVIVING INTESTINE.—Continued

No. of Experiment.	Agent	End Concentration in Ringer's Solution, per Cent.	Species	Effect on Peristalsis	Kind of Strip
			Nuclein Solution (Abbott)		
165	Nuclein solution (fresh)	2.0	Rabbit	Momentary abolition of peristalsis with gradual and prolonged recovery	Longitudinal
166	Nuclein solution (fresh)	2.0	Rabbit	Lasting continual depression (lessened tone); recovery on removal of nuclein	Longitudinal
			Althea Extract (15 per cent.)		
165	Althea extract (slightly acid)	0.3	Rabbit	Gradual depression (lessened amplitude)	Longitudinal
165	Althea extract (slightly alkaline)	0.3	Rabbit	None	Longitudinal
166	Althea extract (slightly acid; natural)	2.0	Rabbit	Abolition of peristalsis; recovery on removal of althea	Longitudinal
166	Althea extract (slightly acid; natural)	0.2	Rabbit	Gradual depression and abolition (lessened amplitude) recovery on removal of althea	Longitudinal
166	Althea extract (neutral)	0.2	Rabbit	Momentary depression followed by stimulation	Longitudinal
166	Althea extract (neutral)	1.0	Rabbit	Marked lasting stimulation (increased tone and amplitude)	Longitudinal
166	Althea extract (neutral)	1.0	Rabbit	Momentary depression, followed by considerable lasting stimulation (increased amplitude)	Longitudinal
166	Althea extract (neutral)	2.0	Rabbit	Marked prompt stimulation (increased tone and amplitude)	Longitudinal
	Althea extract (neutral)	1.0	Rabbit	Prompt moderate stimulation (increased tone and amplitude)	Longitudinal
166	Althea extract (neutral)	1.0	Rabbit	Prompt moderate stimulation (increased tone and amplitude)	Longitudinal
166	Althea extract (very slightly acid; natural)	1.0	Rabbit	Gradual temporary depression (lessened tone and amplitude)	Longitudinal
166	Althea extract (very slightly acid; natural)	2.0	Rabbit	Moderate gradual, lasting stimulation (increased amplitude)	Longitudinal
	Althea extract (very slightly acid; natural)	1.0	Rabbit	Moderate stimulation (lessened tone, increased amplitude)	Longitudinal
			Agar sol (0.5 per cent.) and Agar sol gel (1 : 6)		
166	Agar sol	0.02	Rabbit	None	Longitudinal
166	Agar sol	0.05	Rabbit	None	Longitudinal
166	Agar sol	0.10	Rabbit	Slight depression (slowed rate)	Longitudinal
70	Agar sol	0.01	Guinea-pig	None	Longitudinal
70	Agar sol	0.05	Guinea-pig	Slight depression (slowed rate)	Longitudinal
73	Agar sol	0.02	Guinea-pig	Slight depression (slowed rate)	Longitudinal
73	Agar sol gel	0.017	Guinea-pig	None	Longitudinal
73	Agar sol gel	0.017	Guinea-pig	None	Longitudinal
73	Agar sol gel	0.017	Guinea-pig	None	Circular

would be generally expected with colloids, that is, a limitation of physiologic activity because of their peculiar physical chemical properties (lessened diffusion and dissociation of ions, absorption, etc.) with effects on nutrition.

As far as acacia is concerned, my results are confirmative of a preliminary report by Kruse.[2] Accordingly, the use of acacia intravenously can not be regarded without the possibilities of causing injury, or at least effects that are not necessarily beneficial.

The results reported in this paper are in line with those on bronchial musculature described in previous papers, and prove conclusively that the disturbances produced by the intravenous injection of these and certain other agents such as arsphenamin, bear no relationship whatsoever to anaphylaxis or anaphylactic shock.

1. The direct application of agar sol and agar sol-gel to surviving intestine and uterus produces either no effect or slight depression of peristalsis.

2. The following agents in high and low concentrations uniformly depressed the peristalsis of surviving intestine and uterus: acacia, dextrin, glycogen, gelatin, starch, human and horse serums and nuclein solution. Althea extract was somewhat variable, although depression was most common, irrespective of the chemical reaction.

3. The following agents used as controls produced moderate to marked stimulation of intestinal and uterine peristalsis: peptone and rabbit's serum. Beef serum was irregular. Definite and rather marked stimulation was produced by congo red in low concentrations. This does not agree with the results obtained on the bronchi of perfused lungs.

4. These effects on intestinal and uterine musculature agree with those on bronchial musculature (except congo red) previously reported.

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[2] Kruse: Am. J. Physiol. **49**: 137 (Proc.) 1919.

TABLE 2.—EFFECTS OF PEPTONE, SERUM AND VARIOUS NONPROTEIN COLLOIDS ON SURVIVING UTERUS OF RABBIT (LONGITUDINAL STRIPS)

Number of Experiment	Agent	End Concentration in Ringer's Solution, per Cent.	Effect on Peristalsis
		Peptone (10 per cent.)	
165	Peptone	0.2	Stimulation (increased rate and amplitude)
165	Peptone	0.4	Further stimulation
167	Peptone	0.03	Moderate stimulation (increased amplitude and rate)
167	Peptone	1.0	Marked lasting stimulation (increased tone and rate at first; later, lessened, increased amplitude)
		Acacia (6 per cent)	
167	Acacia	1.0	Depression more lasting (lessened tone and amplitude)
167	Acacia	1.2	Prompt marked lasting depression; gradual recovery (chiefly lessened tone)
		Dextrin (6 per cent.)	
167	Dextrin	1.2	Depression; gradual recovery (lessened tone and amplitude)
		Glycogen (2.5 per cent.)	
165	Glycogen	0.15	Depression (lessened amplitude)
167	Glycogen	0.1	Depression (lessened tone and amplitude)
167	Glycogen	0.1	Moderate temporary depression
		Gelatin (6 per cent.)	
165	Gelatin	1.2	None
167	Gelatin	0.48	Marked temporary depression (lessened tone and amplitude)
167	Gelatin	1.2	Prompt lasting marked depression; (lessened tone and amplitude); gradual recovery
		Soluble Starch (6 per cent.)	
165	Starch	1.2	None
167	Starch	1.2	Gradual temporary depression (lessened tone and amplitude)

		Serums (whole)	
165	Human (old, preserved with trikresol)	4.0	None
167	Human (old, preserved with trikresol)	8.0	Gradual depression (lessened tone); recovery on removal of serum
167	Human (old; preserved with toluol removed)	2.0	Gradual depression (lessened tone); recovery on removal of serum
167	Rabbit (old; preserved with trikresol)	2.0	Marked deprression; no recovery (lessened tone and amplitude)
167	Rabbit (old; preserved with trikresol)	2.0	Further depression
167	Rabbit (old; dialyzed; undecomposed)	2.0	Considerable lasting stimulation (increased tone, amplitude and rate)
165	Beef (old; preserved with trikresol)	4.0	None
167	Beef (old; no preservative; undecomposed)	2.0	Stimulation (prevention of falling tone; diminished amplitude)
167	Beef dialyzed	2.0	Depression (loss of tone and amplitude); recovery on removal
		Congo Red (1 per cent.)	
165	Congo red	0.04	Very slight stimulation (increased amplitude)
167	Congo red	0.04	Considerable stimulation (increased tone and amplitude)
167	Congo red	0.2	Lasting stimulation (increased tone)
		Nuclein Solution (Abbott)	
165	Nuclein solution	3.0	None
165	Nuclein solution	1.0	Considerable depression (lessened tone and amplitude)
165	Nuclein solution	2.0	Considerable depression (gradual recovery)
		Althea extract (10 per cent. and 15 per cent.)	
165	Althea extract (15 per cent.; slightly acid)	1.5	Depression
167	Althea extract (10 per cent.; neutral)	2.0	Stimulation (gradual increase in tone and amplitude)
167	Althea extract (10 per cent.; slightly acid; natural)	2.0	Marked depression and temporary abolition of peristalsis (lessened tone, and amplitude; gradual recovery)
		Agar (0.5 per cent.)	
165	Agar sol	0.02	None
165	Agar sol	0.02	None
167	Agar sol	0.1	Temporary depression; prompt recovery (lessened amplitude)
		Pollen Extract (0.01 mg. nitrogen to 1 c.c.; Mulford's)	
167	Hay fever spring	2.0	Depression (lessened tone)
167	Hay fever fall	2.0	Depression (lessened tone)
		Trikresol (0.3 per cent.)	
167	Trikresol	0.006	Prompt marked depression (lessened tone); gradual recovery; complete recovery on removal of trikresol

5. The results of this study sustain the contention elaborated in previous papers as to bronchial musculature, that the disturbances produced by the intravenous injection of agar and various nonprotein colloids, and also arsphenamin, bear no relationship whatsoever to anaphylaxis or anaphylactic shock.

HEMAGGLUTINATION IN VITRO BY AGENTS WHICH PRODUCE ANAPHYLACTOID SYMPTOMS *

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(From The Journal of Pharmacology and Experimental Therapeutics, February, 1920, p. 479)

INTRODUCTION

Certain of the colloid and other agents described in previous papers [1] [2] by us were found to produce pulmonary hemorrhages and thrombi in the pulmonary capillaries after intravenous injection in guinea-pigs. Among these agar and acacia were found to produce these phenomena most constantly. The marked pulmonary distention produced by agar is attributed to passive bronchoconstriction produced by the thrombi and emboli in both intact and perfused lungs. With acacia and the remaining agents the thrombi, which were found in intact lungs, did not occur in perfused surviving lungs; and distention of these was variable or absent. Hence, the mechanism of thrombus formation in intact lungs must be regarded as of considerable importance, particularly with agents which are used intravenously for therapeutic purposes such as acacia, certain thromboplastic agents, arsphenamin, peptone, etc. Conglutination of the corpuscles is conceivable in the formation of these thrombi.

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* Received for publication Oct. 31, 1919.

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[1] Hanzlik, Karsner and Fetterman: J. Pharm. Exp. Therap. **14**: 229.

[2] Hanzlik and Karsner: J. Pharm. Exp. Therap. **14**: 379, 425.

Agglutination of cat corpuscles by acacia in vitro has been observed by Bayliss,[3] reported by Robertson[4] with cat but not human corpuscles, and by Kruse[5] with human, dog, cat and rabbit, but not with ox, frog and turtle corpuscles. Other agents that were studied by us besides acacia, namely, agar, thromboplastin, coagulen, arsenicals, etc., were found to produce pulmonary thrombi and emboli and even more severe anaphylactoid symptoms. It is reasonable to suppose that these might cause agglutination even more strikingly than acacia. It was, therefore, resolved to investigate the entire series of colloid and other agents used in our previous studies on anaphylactoid symptoms with the hope that the results might further elucidate the accidents of intravenous medication and the causation of thrombi where these have been observed, although, of course, agglutination in vitro does not necessarily mean that the same occurs in vivo.

METHOD

Fresh human, cat and guinea-pig red blood corpuscles were washed three times with salt solution (0.9 per cent. NaCl usually, but 0.85 per cent. in a few of the tests) and made up to a 5 per cent. suspension of the cells. One cubic centimeter of blood suspension was mixed with 1 c.c. of the agent to be tested so that the final blood suspension was 2.5 per cent. The agents tested were in such concentration that the final mixture in the test tubes produced the desired end concentrations. These are indicated in the tables. Readings were made after two hours in a water bath at 38 C., and again after twenty-two hours additional in the refrigerator. Record was made not only of agglutination but also of hemolysis and precipitation.

CONCENTRATION OF AGENTS

At least two concentrations in the majority of tests were used: (1) Very low concentrations such as occur after the intravenous administration of certain of the agents therapeutically in human individuals; (2) Higher, and also low concentrations, which were used in our previous experiments on guinea-pigs with agents causing anaphylactoid symptoms. These concentrations were calculated on the basis of blood being equivalent to 6 per cent. of body weight, which was taken as 300 grams for the average guinea-pig in our former experiments. In the case of man 60 kgm. was taken as the average weight. The concentrations in cat's blood were the same as in guinea-pig's and human blood.

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[3] Bayliss: Intravenous Injection in Wound Shock, 1918, p. 172, Longmans, Green & Co.

[4] Robertson, O. H.: Central Med. Dept. Lab. Div. of Surg. Research A. P. O. 721, France, Oct. 27, 1918.

[5] Kruse: Am. J. Physiol. **49**: 137 (Proc.) 1919.

In addition, the lowest effective concentrations of those agents, which proved to be agglutinators, were ascertained as closely as possible.

RESULTS

All of the results that were obtained with the thirty-one different agents tested are presented in Tables 1, 2, 3 and 4. These are further correlated in Tables 5, 6 and 7 with the results, previously reported,[2] and pertaining to pulmonary thrombi and hemorrhages after the injection of the same agents in guinea-pigs.

AGGLUTINATION AND THROMBOSIS

In interpreting and attempting to correlate the production of agglutination in vitro and thrombosis in vivo there are certain points which must be given consideration. Thrombosis occasionally but rarely occurs after normal salt solution injections as seen in one of our controls. Conglutination of corpuscles may occur as the result of fixation or of decomposition of tissues before fixation. In the present series, the lung tissues were cut into blocks not more than 4 mm. thick, placed in carefully prepared Zenker fluid not more than five minutes after the death of the animal, using two or three blocks in 30 to 50 c.c. fluid. At the end of twenty-four hours the blocks were washed in freely running water for twenty-four hours and the usual paraffin method followed. As nearly as possible the procedure was uniform throughout. Therefore, the

TABLE 1.—AGGLUTINATING AGENTS *

Agent	End Concentration, per Cent.	Human Cells		Cat Cells		Guinea-Pig Cells	
		2 Hrs.	24 Hrs.	2 Hrs.	24 Hrs.	2 Hrs.	24 Hrs.
Acacia (Khardofan)....	1.0 (2)	+	+	+	+	+	+
	0.8	—	—	—	—	—	—
	0.5	—	—	—	—	—	—
	0.1 (2)	—	—	—	—	—	—
	0.05 (2)	—	—	—	—	—	—
	0.016	—	—	—	—	—	—
Althea......	1.2	+	+	+	+	+	+
	0.62	±	+	+	+	+	+
	0.31	+	+	+	+	+	+
	0.15	±	+	+	+	+	+
	0.08	+	+	+	+	+	+
	0.05	—	—	—	—	—	—
	0.01	—	—	—	—	—	—
	0.005	—	—	—	—	—	—
Agar....................	0.055	+	+	+	+	+	+
Agar sol gel †...........	0.002	+	+	+	+	+	+
	0.001	±	+	±	+	±	+
	0.001	+	—	+	—	+	—
	0.0005	—	—	—	—	—	—
	0.00025	—	—	—	—	—	—
	0.00012	—	—	—	—	—	—
Arsphenamin ‡..........	0.166	+	+	+	CH	+	CH
	0.083	+	+	+	CH	+	+
	0.04	+	+	+	+	+	+
Atropin sulphate........	0.03 (2)	+	+	+	+	+	+
	0.015	+	+	+	+	+	+
	0.005	±	+	±	+	±	+
	0.002	—	+	—	±	—	—
Beef serum..............	.....	—	+	—	+	—	+
Collargol................	0.17	+	+	+	+	+	+
	0.011	—	—	—	—	—	—
	0.011	+	+	+	+	+	+
	0.005	+	+	+	+	+	+
Gelatin.....	1.0	+	+	+	+	+	+
	0.5	+	+	+	+	±	+
	0.1 (2)	+	+	+	+	+	+
	0.05	+	+	+	+	+	+
	0.01	—	—	—	—	—	—
	0.005	—	—	—	—	—	—
Neo-arsphenamin.......	0.025 (ppt.)	+	+	+	+	+	+
	0.025	+	+	+	+	+	+
	0.025	—	—	—	—	+	+
Nuclein..................	10.0 (2)	—	+	—	+	—	+
	5.0	—	+	—	+	—	+
	0.6	—	+	—	+	—	+
Soluble starch...........	1.0 (3)	+	+	+	+	+	+
	0.5	+	+	+	+	+	+
	0.5	+	+	—	+	—	+
	0.1	—	—	—	—	—	—
	0.05	—	—	—	—	—	
	0.01	—	—	—	—	—	—
Thromboplastin (Squibb)	17.0	+	+	+	+	+	+
	17.0	—	—	—	—	—	—
	2.8	+	+	+	+	+	+
	2.8	—	+	—	+	—	—
Tragacanth.............	0.05	+	+	+	+	+	+
Salt solution (0.9 per cent. NaCl: control)		—	—	—	—	—	—

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* The various signs and abbreviations have meanings as follows: (+) = agglutination present; (—) = agglutination absent; CH = complete hemolysis; figures in parentheses denote number of tests.

† Prepared according to the method of Novy and DeKruif: J. Inf. Dis , 1917, **20**: 629.

‡ From Dermatological Research Laboratories.

series served as its own control, and conglutination occurred very rarely following the injection of agents which otherwise did not produce thrombosis, namely, beef serum, hemostatic serum, althea, pollen extract, phylacogen and peptone. In previous studies by one of us (H. T. K.) it was shown that conglutination is common following the injection of beef serum and of peptone. We feel that in the present series it is unwise to lay much stress on conglutination of erythrocytes unless the agent employed has produced definite thrombosis with platelet and fibrin deposition. Table 5 gives the results of intravenous injection of agglutinating substances. The results as to thrombosis and conglutination were reported in a previous paper of this series.

TABLE 2.—AGENTS FAILING TO PRODUCE AGGLUTINATION

Agent	Per Cent.	Agent
Coagulen (Ciba).....	0.5	Peptone, 0.002 per cent.
Coagulen (Ciba).....	0.1	Pollen extract,‡ hay fever fall, 6 per cent. (2)*
Congo red...........	0.34	Pollen extract,‡ hay fever spring, 6 per cent. (2)
Congo red...........	0.17	Serum, dog, 11 per cent.
Dextrin..............	1.0	Serum, horse, 11 per cent.
Epinephrin...........	0.000083	Serum, human, 11 per cent.
Glycogen.............	0.1	Serum, rabbit, 11 per cent.
Glycogen†............	0.05	Sodium arsenate, 0.013 per cent.
		Sodium chlorid, 0.9 per cent.
Kephalin†............	0.017	Trikresol, 0.05 per cent.
Peptone (Witte).....	1.0	Venarsen,§ 0.26 per cent.

* Figures in parentheses indicate number of tests.
† Prepared from pig's brains according to method of Howell.
‡ Furnished by H. K. Mulford and Company, Philadelphia.
§ Principal constituent is sodium cacodylate.

Of these agents it will be seen that there appears to be a definite correlation between agglutination and thrombosis in the case of acacia, agar, arsphenamin, beef serum, collargol and thromboplastin, or in one-half the agents which caused agglutination in vitro. Nevertheless, such marked agglutinators as althea, gelatin and starch failed to produce thrombosis except for one animal in the althea series which showed conglutination of corpuscles.

Atropin was used in conjunction with numerous agents and thrombosis appears in connection with agar (two animals), arsphenamin (two animals, one of which shows only conglutination), typhobacterin (one animal), pollen extract (one animal showing conglutination). Of these agents agar and arsphenamin were commonly associated with thrombus formation and it may safely be stated that in spite of the surprising and marked agglutination in the test tube produced by atropin sulphate, large intravenous doses failed to produce thrombosis.

TABLE 3.—AGENTS PRODUCING HEMOLYSIS *

Agent	End Concentration, per Cent.	Human Cells		Cat Cells		Guinea-Pig Cells	
		2 Hrs.	24 Hrs.	2 Hrs.	24 Hrs.	2 Hrs.	24 Hrs.
Acacia........................	1.0	—	—	—	—	—	P
	0.5	—	—	—	—	—	P
Arsphenamin..............	0.166	—	—	P	C	P	C
	0.083	S	P	P	C	P	P
Coagulen (Ciba)..........	0.5 (2)	—	P	—	P	—	P
	0.1	—	S	—	S	—	S
	0.1	—	—	—	S	—	P
Collargol....................	0.011 (2)	—	P	—	P	—	P
Gelatin.......................	2.5	—	P	—	S	—	P
	1.0	—	—	—	—	—	—
Kephalin.....................	0.05	—	S	—	P	—	P
	0.017	—	—	—	S	—	P
Nuclein solution...........	0.6	—	—	—	—	—	P
Serum, dog..................	.....	—	P	—	P	P	C
Thromboplastin (Squibb)	17.0 (2)	—	P	—	P	—	P
	2.8 (2)	—	P	—	P		P
Thromboplastin (Armour)	17.0	—	P	—	P	—	P
Salt solution (0.9 % NaCl)	(6)	—	—	—	—	—	—

* The signs and abbreviations in this table have meanings as follows: — = no hemolysis; S = slight hemolysis; P = partial hemolysis; C = complete hemolysis.

In this connection it should be remembered that the solutions of atropin (particularly the higher concentrations) were acid. This is also true of althea extract. The agglutination in vitro, at least with high concentrations, can be explained by this, but when injected intravenously the acidity is quickly neutralized by the reserve alkali unless injected in very large quantities. The hydrogen ion concentration of the mixtures was not estimated. On the other hand, with such agents as agar, acacia, collargol, gelatin, beef serum, neo-arsphenamin and starch, whose chemical reaction is practically neutral, the mechanism responsible for the agglutination (in vitro) must be different. Arsphenamin solutions are, of course, slightly alkaline (also serum), and how this would affect agglutination we do not know.

Of the fifteen agents which failed to produce agglutination in the test tube the following five (coagulen, kephalin, pollen extract, human serum and sodium arsenate) produced thrombosis in the animals according to the summary in Table 6. The results of the injection experiments have been previously reported.

TABLE 4.—AGENTS PRODUCING DARK BROWN DISCOLORATION OF BLOOD

Agent	End Concentration, per Cent.	Human Cells		Cat Cells		Guinea-Pig Cells	
		2 Hrs.	24 Hrs.	2 Hrs.	24 Hrs.	2 Hrs.	24 Hrs.
Althea	2.5	+	+	+	+	+	+
Arsphenamin	0.166	+	+	+	+	+	+
	0.083	+	+	+	+	+	+
	0.04	—	—	+	+	+	+
Geltain	2.5	—	—	—	—	+	+
	1.0	—	—	—	—	+	+
	1.0	+	+	+	+	+	+
	0.1	+	+	+	+	+	+
Neo-arsphenamin	0.023 (ppt.)	+	+	+	+	+	+
	0.023	+	+	+	+	+	+
	0.023	—	—	+	+	+	+
Peptone	1.0	+	+	+	+	+	+
Soluble starch	1.0	—	+	—	+	—	+
	0.5	—	+	—	+	—	+
Trikresol	0.05	+	+	+	+	+	+
Salt solution	.....	—	—	—	—	—	—

The signs and abbreviations have meanings as follows: + = discoloration present; — = discoloration absent; ppt. = precipitate.

Thus it will be seen that only a small number of the agents which are nonagglutinators show thrombosis and only three of these (kephalin, human serum and sodium arsenate) show any important relationship.

Of the nonagglutinators, epinephrin deserves separate comment. It was used in ten animals in conjunction with other agents and in four animals there was thrombosis. Two of these four were injected with agglutinating and thrombus producing agents, namely, agar and arsphenamin. The other two were injected with peptone and with horse serum (anaphylaxis), both animals showing only conglutination of corpuscles. Hence epinephrin is included among those agents which produced neither agglutination in the test tube nor thrombosis in the animals.

As far as the results with agglutination in vitro are concerned, these are confirmative of those of Ono[6] for gelatin, acacia, tragacanth and althea, and of Kruse[5] for acacia. The concentrations used by Ono and Kruse are not known to us. The results obtained by us with gelatin and acacia indicate that they can agglutinate human corpuscles with concentrations corresponding to those occurring in the intravenous administration of these as therapeutic agents in traumatic shock, hemorrhage, etc. This, taken together with the fact that

TABLE 5

Agglutinating Agents	Total Number of Animals Injected	Number of Animals Showing Thrombosis	Number of Animals Showing Conglutination
Acacia	10	3	3
Agar	14	7	..
Althea	3	..	1
Arsphenamin	6	2	2
Beef serum	2	..	1
Collargol	3	1	1
Gelatin	2	..	..
Neo-arsphenamin	3	..	..
Nuclein	3	..	..
Soluble starch	3	..	..
Thromboplastin (Squibb)	5	1	2
Atropin sulphate (with other agents)	21	4	2

TABLE 6

Nonagglutinating Agents	Total Number of Animals Injected	Number of Animals Showing Thrombosis	Number of Animals Showing Conglutination
Coagulen	8	1	1
Kephalin	4	2	..
Pollen extract	7	..	3
Human serum	1	1	..
Sodium arsenate	2	1	..

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[6] Ono: Acta Scholae Med. Univ. Imp. Kioto **2**: 271, 1918; abst., Chem. Absts. **13**: 1098, 1919; abst., J. A. M. A. **71**: 502, 1918.

acacia forms pulmonary thrombi in guinea-pigs, indicates that the use of acacia intravenously should be contemplated with definite possibilities of causing injury.

Concerning the mechanism of action of the agglutinating agents, the evidences which we have are insufficient to permit an analysis of this. Most of the agents that acted as agglutinators are colloids. However, the physical properties of certain of these are too different to permit of any general classification. For instance, acacia and gelatin in the concentration of 10 to 12 grams per liter possess osmotic pressures of 72 and 6 (mm. Hg) and molar weights of 2,400 and 36,000 (Taylor, W.[7]), respectively, yet both acted as agglutinators; gelatin somewhat more effectively. Other colloidal agents (see Table 6) again did not agglutinate. Finally, atropin, acids and other agents which behave like crystalloids also agglutinate. A colloidal state, therefore, does not seem to be indispensable for agglutination. The relation is most likely to be sought in the electric charges of the colloids.

HEMOLYSIS AND HEMORRHAGE

From the data in Table 3, it is seen that the most notable hemolyzing agent was arsphenamin, which affected cat and guinea-pig cells more markedly than human cells. The least active of this group were acacia, nuclein and gelatin, acacia and gelatin affecting particularly the guinea-pig cells. The others ranged as hemolyzing agents of only moderate activity. It is to be noted that none of the agents completely hemolyzed human blood and only a few of the other bloods employed. The conditions in the test tube were arranged with the primary idea of testing for agglutination and were not ideal for hemolysis. It is possible that the presence of complement might have made a difference in the completeness of hemolysis but it is unlikely that other important variations from the result shown would have occurred. Table 7 shows the attempt at correlation between test tube hemolysis and pulmonary hemorrhage.

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[7] Taylor, E. W.: Chemistry of Colloids, 1915, pp. 3-8. Longmans, Green & Co.

Dog serum might have been included in the above table but for the fact that no animals were injected in this series. It is well known, however, that dog serum is toxic for guinea-pigs and produces hemorrhage in the lungs of injected animals. Table 2 shows that it produced hemolysis in all the bloods employed, particularly that of the guinea-pig.

TABLE 7

Hemolytic Agents	Total Number of Animals Injected	Pulmonary Hemorrhage Present in
Acacia	10	3
Arsphenamin	6	6
Coagulen (Ciba)	8	3
Collargol	3	1
Gelatin	2	2
Kephalin	4	2
Nuclein solution (Abbott)	3	2
Thromboplastin (Squibb)	5	4

The hemolytic agents which produced hemorrhages in all animals injected are arsphenamin (alkaline) and gelatin, both of which also produced discoloration of the blood. Thromboplastin and nuclein solution produced hemorrhages in a high percentage of animals. The other four hemolytic agents, namely, acacia, coagulen, collargol and kephalin, produced hemorrhages in only a relatively small percentage of the animals employed.

Thirteen other agents, namely, althea, agar, beef serum, neo-arsphenamin, starch, congo red, dextrin, peptone, pollen extract, human serum, rabbit serum, sodium arsenate and venarsen, produced no hemolysis, but distinct hemorrhages, and four agents (glycogen, horse serum, trigresol and normal saline) produced neither hemolysis nor distinct hemorrhage.

BLOOD DISCOLORATION

The data in Table 4 indicate that discoloration of the blood was most markedly displayed by arsphenamin, a property also exhibited in slightly less degree by neo-arsphenamin. As with hemolysis, blood discoloration was somewhat less marked with human blood than with cat and guinea-pig blood.

To what extent the discoloration of the blood that was observed was due to methemoglobin alone can not be said definitely without more definite analysis than by direct inspection alone. A part of the discoloration was no doubt due to the formation of acid and alkali hematins from the following agents which gave evidences of definite chemical reaction toward litmus; althea extract (acid) and arsphenamin (alkaline). The formation of acid and alkali hematins can not be invoked for the following agents which were practically neutral in reaction; neo-arsphenamin, gelatin, peptone, starch and trikresol. The discoloration from these agents may be regarded as due to methemoglobin with greater certainty. Unfortunately, the hydrogen ion concentration of the different mixtures was not ascertained. The incidence of blood discoloration and pulmonary hemorrhage was too variable to warrant the drawing of definite conclusions.

CONCLUSIONS

1. The following agents and their approximate lowest effective end concentrations cause agglutination of human, cat and guinea-pig red blood corpuscles in vitro; acacia (1 per cent.), althea (0.08 per cent.), agar (0.001 per cent.), arsphenamin (<0.04 per cent.), atropin sulphate (0.002 per cent.), beef serum collargol (<0.005 per cent.), gelatin (0.05 per cent.) neo-arsphenamin (0.025 per cent.), nuclein solution, starch (0.5 per cent.), thromboplastin (Squibb, 2.8 per cent.) and tragacanth.

2. No demonstrable agglutination was produced by the following: coagulen, (0.5 per cent.), congo red (0.34 per cent.), dextrin (1 per cent), epinephrin (0.000083 per cent.), glycogen (0.1 per cent.), kephalin (0.05 per cent.), peptone (Witte, 1 per cent.), pollen extracts (6 per cent.), serums of dog, horse,

man and rabbit (11 per cent.), sodium arsenate (0.05 per cent.), venarsen (0.26 per cent.), and normal saline (0.9 per cent. NaCl).

3. Hemolysis though variable was produced by acacia, arsphenamin, coagulen, collargol, gelatin, kephalin, nuclein solution, dog serum and thromboplastin (Squibb and Armour), and blood discoloration (methemoglobin and hematin formation, etc.) by althea, arsphenamin, gelatin, neo-arsphenamin, peptone, starch, and trikresol.

4. Agglutination in vitro is a common phenomenon among agents which produce anaphylactoid symptoms.

5. Hemagglutination in vitro and thrombus formation are closely correlated in certain instances, but this relation is by no means constant or necessary.

6. Those agents which cause hemolysis in vitro are frequently followed by pulmonary hemorrhage after injection in guinea-pigs. On the other hand, hemorrhage frequently occurred with many agents that did not cause hemolysis under the conditions.

THE LIBERATION OF FORMALDEHYD FROM AND DECOMPOSITION OF ANHYDROMETHYLENECITRIC ACID AND ITS EXCRETION IN URINE, WITH COMMENTS ON "CITARIN" AND "HELMITOL" *

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(From The Journal of Urology, April, 1920, p. 145)

INTRODUCTION

Anhydromethylenecitric acid is of interest as a urinary, and possibly as an intestinal, antiseptic because of the possibility of liberating formadehyd from it in alkaline media. The opposite of this is hexamethylenamin, which liberates formaldehyd in fluids possessing a truly acid reaction, i. e., for practical purposes, in acid urine only. By means of these two compounds, therefore, it might appear that the antiseptic armamentarium would be able to meet all the exigencies pertinent to the variabilities in chemical reactions of body fluids. The mechanism of action of hexamethylenamin is well established. Concerning the exact behavior of anhydromethylenecitric acid practically nothing is known, although it has formed the basis of two other products, namely, its sodium salt ("citarin") and the hexamethylenamin anhydromethylenecitrate ("helmitol"), which have been extolled as efficient urinary antiseptics, particularly "helmitol." As far as I am able to ascertain there are only the few and uncritical experiments by Impens [1] on the excretion in urine of man, and on blood pressure in cats. The advantages that "helmitol" is alleged to possess over hexamethylenamin are

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* From the Pharmacological Laboratory, School of Medicine, Western Reserve University, Cleveland.

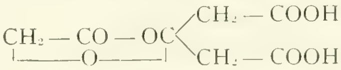
* Received for publication Jan. 25, 1920.

[1] Impens: Monatschf. f. Urologie **8**: 257, 1903; Deutsch. Arch. f. Klin. Med. **82**: 409, 1905.

ascribed to its anhydromethylenecitric acid component. Obviously, such claims in the absence of definite knowledge concerning the behavior of anhydromethylenecitric acid are pure speculation and unjustified. Therefore, it seemed appropriate to investigate the behavior of anhydromethylenecitric acid. .

The experiments were planned to determine mainly the exact degree of alkalinity under which liberation of formaldehyd occurs; the decomposition of anhydromethylenecitric acid; its excretion in urine; liberation of formaldehyd in ammoniacal urine and preservation of urine. A few subsidiary points were also elucidated. Before proceeding to the results a brief description of anhydromethylenecitric acid is desirable.

Description.—Anhydromethylenecitric acid is a white, crystalline solid of rather strong acid taste; melting point, 208 F. Its solubility is relatively slow in water and acids, more rapid in alkalies with the liberation of formaldehyd. Its empirical formula is $C_7H_8O_7$ and the structural formula is given by Percy May[2] and by Abderhalden in the Biochemisches Handlexikon[3] as



According to this the molecular weight is 204. The acid is prepared by heating citric acid with paraformaldehyd, or by the action of chlormethyl alcohol ($Cl.CH_2OH$) on citric acid at 130 F. to 140 F. in which case a better yield is obtained.

METHODS

These will be described under the various headings throughout the text.

RESULTS

Solubility.—As indicated by titration with decinormal bicarbonate and hydroxid, using methyl red and methyl orange as indicators, the limit of solubility for anhydromethylenecitric acid in water at room temperature is about 2.3 per cent. One per cent. of acid dissolves slowly and completely with shaking. This is somewhat facilitated by warming, but a high degree of heat must be avoided, since this will liberate formaldehyd. Such solutions would give false results when used for testing as to liberation of formaldehyd. The acid is soluble in alcohol and ether; more freely in alkalies than water and not so readily in dilute acids.

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[2] May, Percy: Chemistry of Synthetic Drugs, 1911, p. 208, Longmans.

[3] Abderhalden: Biochemisches Handlexikon **1**: 1181, 1908.

As far as solubility is concerned it appears that anhydromethylenecitric acid could be relatively easily absorbed from the alimentary tract.

Total Acidity.—One-hundredth gram of anhydromethylenecitric acid (i. e., 1 c.c. of 1 per cent. in water) was found to require 1.04 c.c. of N/10 NaOH (= to 1.03 c.c. N/10 HCl) or N/10 $NaHCO_3$, using methyl red and methyl orange as indicators. That is, 0.01 gram of the acid requires about 0.0043 gram of NaOH or 0.0089 gram of $NaHCO_3$ for neutralization. One-hundredth gram of the acid was found to require 0.00502 gram $(NH_4)_2CO_3$ for neutralization. Whether the concentrations of alkaline salts represented by these solutions would suffice for the liberation of formaldehyd is another question. This would depend on the degree of alkalinity, i.e., hydroxyl ion concentration rather than the concentration of the alkaline salts. This will be considered later. The concentration of bicarbonate (0.6 per cent.) necessary for neutralization of 1 per cent. anhydromethylenecitric acid may occur in intestinal juice. Since no demonstrable formaldehyd is liberated at neutrality, it is entirely conceivable that the anhydromethylenecitrate is absorbed as such from the alimentary tract without decomposition. This proved to be the case, in part at least, as indicated by the data to be referred to later.

Free Acidity.—Anhydromethylenecitric acid possesses a rather strong acid taste, although as compared with strong mineral acids, it is a relatively weak acid. The hydrogen ion concentration of a 1 per cent. solution was found to be about p_H 2.4, using methyl violet as an indicator, or, in other words, the acidity of about a normal (N/1) solution of acetic acid. This is much weaker than the acidity of N/10 HCl, which gives a p_H of about 1.0.

Accordingly, anhydromethylenecitric acid should be rather irritating, particularly if used in large doses.

Free and Total Acidity of "Helmitol."—"Helmitol" possesses a distinctly acid taste, which is lost when the drug is completely neutralized by hexamethylenamin. The hydrogen ion concentration of a 1 per cent. solution was found to be somewhat weaker than that of anhydromethylenecitric acid, namely between p_H 3.8 and 4.0, using methyl violet as an indicator.

One-hundredth gram of "helmitol" was found to require 0.4 c.c. of N/10 sodium hydroxid for neutralization or about 0.4 of that for a corresponding quantity of anhydromethylenecitric acid. In other words, 1 part of "helmitol" appears to contain about 0.4 part, or roughly one-half, of anhydromethylenecitric acid. Since anhydromethylenecitric acid possesses two carboxyl groups, it would appear that "helmitol" is only about half neutralized by the base, hexamethylenamin. This would make it an acid salt.

However, actual titration with hexamethylenamin using different indicators gives peculiar results. Using methyl red as an indicator, it was found that about 0.086 to 0.09 gram of hexamethylenamin is necessary for the neutralization of 0.01 gram of anhydromethylenecitric acid. Theoretically, 0.01 gram of anhydromethylenecitric acid should require 0.0137 gram of hexamethylenamin, providing both hydrogen atoms of the two caboxyl groups are replaced by hexamethylenamin. The quantity found necessary by me was about six times the theoretical. Using methyl orange as an indicator, the same quantity (0.01 gram) of anhydromethylenecitric acid required only 0.013 gram hexamethylenamin, which is equivalent to 100 per cent. of theory, but the solution was still acid to methyl red, and phenolsulphonephthalein. That is, complete neutrality is not attained when methyl orange is used. With congo red as an indicator, the same quantity (0.01 gram) of the acid required 0.025 gram of hexamethylenamin, which is again too high as compared with the theoretic requirement. "Helmitol" itself is acid to congo red. In these titrations considerable difficulty was experienced in recognizing the end-points, particularly with methyl red and congo red. Therefore, it is probable that under these conditions it is impossible to arrive accurately at the neutralizing power of hexamethylenamin. It is interesting to note that the solutions titrated with hexamethylenamin, using methyl red and congo red as indicators, did not possess an acid taste, but were rather salty, and later slightly sweetish. On the other hand, "helmitol," which is said to be a definite chemical compound of hexamethylenamin and anhydromethylenecitric acid, and the titrations in which methyl orange was used, tasted strongly acid. Anhydromethylenecitric acid completely neutralized by sodium hydroxid also did not possess an acid taste.

The results indicate that "helmitol" is an imperfectly neutralized anhydromethylenecitric acid (by hexamethylenamin). This phase of the study was not pursued further in view of the results which later established the worthlessness of anhydromethylenecitric acid as an antiseptic.

Changes in true reaction (p_H) *attained by the addition of different alkalies to anhydromethylenecitric acid.*—When anhydromethylenecitric acid is neutralized by titration with bicarbonate and hydroxid, using methyl red and methyl orange as indicators, it is still acid toward phenolsulphonephthalein. It appeared probable that the liberation of formaldehyd from anhydromethylenecitric acid depends on a definite degree of alkalinity. From the fact that alkali is consumed in neutralization, it was necessary to ascertain the magnitude of changes in p_H obtainable by the addition of alkalies. This was done colorimetrically with phenolsulphonephthalein. Decinormal alkalies (hydroxid, bicarbonate and ammonium carbonate) and phosphate "buffer" mixtures were used. These were added gradually to 0.01 gram of anhydromethylenecitric acid (1 c.c. of 1 per cent.) adding phenolsulphonephthalein as indicator and comparing the final mixtures with a series of standard phosphate mixtures and alkalies ranging from p_H 6.4 to 14.07. It was endeavored to ascertain how much alkali was necessary to bring about an end-reaction approaching that which occurs in alkaline body fluids, such as intestinal juice and blood, and that attainable in urine after the administration of alkali.

TABLE 1.—QUANTITIES OF DIFFERENT ALKALIES NECESSARY TO PRODUCE DIFFERENT CHANGES IN PH OF 0.01 GRAM OF ANHYDROMETHYLENECITRIC ACID (1 C.c. OF 1 PER CENT.)

N/10 $NaHCO_3$		N/10 NaOH		$(NH_4)_2CO_3$ 0.68 N		"Buffer" Phosphate Mixtures	
						pH of Phosphate Mixture Used	End pH of Phosphate and Anhydromethylenecitric Acid Mixture
C.c	pH	C.c.	pH	C.c.	pH		
1.05	6.5	1.05	6.5	1.65	<6.4	7.6	7.0
2.7	7.0	1.11	7.5	2.5	6.6	7.7	7.1
3.0	7.2	1.20	>8.4	2.6	7.0	7.8	7.1
3.12	7.4	2.00	13.07	2.65	7.1	8.0	7.2
3.25	7.6			2.7	7.2	8.2	7.3
3.5	7.8			2.8	7.4	8.4	7.4
3.6	8.3 (?)			2.95	7.5		
3.82	8.4 (?)			3.0	7.7		
12.00	8.2 or 8.3			3.85	7.8		
18.00	8.3 or 8.2			4.3	8.0		
				4.85	8.2		
				4.65	<8.2		
				>7.0	8.4		
					Could not be reached		

These data were intended to be used for the determination of the exact level of p_H at which formaldehyd is liberated from anhydromethylenecitric acid. This will be discussed later. For the present it may be stated that the level of alkalinity at which liberation occurs was found to be rather high. The data which are presented in Table 1, indicate that anhydromethylenecitric acid requires considerable alkali before neutrality and true alkalinity are reached.

If liberation of formaldehyd cannot occur until true alkalinity is reached, it is apparent that a higher concentration of alkaline salts than can occur in such fluids as intestinal juice and urine, perhaps even fermented or ammoniacal urine, is necessary. It would mean that the alkalinity of intestinal juice would have to be higher than it is naturally in order to decompose the acid with the liberation of formaldehyd and prevent its absorption as such. The data indicate that anhydromethylenecitric acid is probably absorbed as such from the intestine without decomposition. Decomposition, of course, may occur later in the circulation and tissues. This was found to be the case as will be shown later.

If anhydromethylenecitric acid is to exhibit its alleged antiseptic properties as a result of the liberated formadehyd in alkaline urine, the data obtained indicate that the alkalinity necessary for this would have to be higher than is probably attainable. This already speaks against the compound as a urinary antiseptic.

Application of Tests for Formaldehyd Liberated from Anhydromethylenecitric Acid.—In attempting to ascertain the exact degree of alkalinity (as judged by changes in hydrogen ion concentration) necessary for the liberation of formaldehyd from anhydromethylenecitric acid, it is obvious that tests which require a high degree of hydroxyl ion concentration are precluded. This is true of both the phloroglucin and phenylhydrazin-nitro-prusside tests. That is, these tests can not be applied directly to the agent, since the alkalinity, which is necessary for positive reactions with formaldehyd, is high enough to decompose the anhydromethylenecitric acid. Phloroglucin was found to require a p_H of 12.13 before a positive test can be obtained with 1 : 100 formaldehyd, its sensitivity of course being at least 10,000 times as great with proper alkalinity. This degree of alkalinity corresponds to the alkalinity of a solution of 0.01 N sodium hydroxid.

The same holds true of the application of the phloroglucin test in studying the relation of degree of acidity to formaldehyd liberation. This is different with a formaldehyd compound like hexamethylenamin, which is not decomposed even in high degrees of alkalinity. Here the phloroglucin is the test of choice, those requiring an acid reaction being prohibited. If anhydromethylenecitric acid were not decomposable in acid media, certain of the formaldehyd tests performed under conditions of acidity might be used. However, all of these, except Denigé's test [4] require a high degree of acidity (concentrated acid) which is sufficient to decompose the anhydromethylenecitric acid. Denigé's reaction was found to possess a low degree of sensitivity, and to react positively with alkalies alone in the absence of formaldehyd. It was, therefore, necessary to adopt a different procedure for the exact study of the relationship of true chemical reaction to liberation of formaldehyd from anhydromethylenecitric acid than by direct application of various tests. It follows from what has been said about the phloroglucin and similar tests that any conclusions that may be drawn concerning the liberation of formaldehyd from anhydromethylenecitric acid in vitro and in urine without due regard for the proper conditions of applying the test are erroneous and unjustified. Such appears to be the case in the work of Impens, who made a few observations on the liberation of formaldehyd in vitro, and excretion of anhydromethylenecitric acid in urine. As far as I know no other work on this compound has been reported to date. The experiments on the liberation of formaldehyd may now be described.

Liberation of Formaldehyd from Anhydromethylenecitric Acid According to Different Degrees of Alkalinity (p_H).—This was determined by aeration of mixtures of "buffer" phosphate solutions, alkalies of definite hydrogen ion concentration and definite quantities of anhydromethylenecitric acid. Owing to the acidity of anhydromethylenecitric acid, the p_H values of the final mixtures were different from the original solutions of known hydrogen ion concentrations, unless the acid was previously neutralized. Both methods were practiced. That is, one series of solutions was made up by direct addition of solutions of known p_H values, and in another series the anhydromethylenecitric acid was first neutralized with sodium hydroxid or bicarbonate. The quantities of these necessary was indicated in the first part of the paper. In both series of experiments the p_H values of the final mixtures were confirmed by matching against standards of the same salts used in making up the solutions of known p_H values and the same dyes. Phenolsulphonephthalein was used for the range from p_H 6.4 to 8.4, cresolphthalein for the range from 8.3 to 9.6, and phenolphthalein for 8.9 to 14, using in each case 5 c.c. of the solution of the dye (usually 1 per cent. in alcohol) to 10 c.c. of the final mixture, and also in the standards.

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[4] Denigé: Merck's Reagenzien Verzeichnis, 1913, p. 81.

The final mixtures containing the anhydromethylenecitric acid were allowed to stand for ten minutes as a rule; in some cases as high as thirty minutes, in order to allow sufficient time for the decomposition in doubtful cases. These were then aerated rapidly for twenty to thirty minutes into 10 c.c. of 0.4 per cent. ammonia water using long test tubes (20 by 180 mm.) for both the ammonia water and the final mixtures.

After aeration was completed, the ammoniated water, which now also contained hexamethylenamin, providing formaldehyd was liberated in the mixtures, was directly acidified in the long test tube with 10 per cent. sulphuric acid, heated to the boiling point to liberate free formaldehyd and cooled in running water. The phloroglucin test was now applied. As a rule 0.5 c.c. of 1 per cent. phloroglucin [reagent (Merck)] in 10 per cent. sodium hydroxid was used to 10 c.c. of solution. If the test was positive, this showed the liberation of free formaldehyd under the conditions of the mixtures containing anhydromethylenecitric acid in solutions of known hydrogen ion concentration.

By this method it is possible to detect the presence of 1 : 1,000,000 of absolute formaldehyd in the solution being aerated. Numerous experiments with formaldehyd in water were made to determine the limit of sensitivity of this method. Solutions of 1 : 1,000,000 gave definite tests, so the limit of sensitivity is probably even less, though not 1 : 10,000,000, which gave negative results. It seemed best to adhere to the concentration of 1 : 1,000,000, which was regarded low enough for the purpose. That is, the concentration of formaldehyd existing in the mixtures described above must be of no practical importance in the absence of positive tests for formaldehyd, when 0.01 gram of anhydromethylenecitric acid theoretically and by experiment yields about 0.00147 gram formaldehyd in 10 to 20 c.c. of solution, or about 1 : 6,800 to 1 : 13,000 concentrations of formaldehyd, respectively. In the majority of my experiments 1 c.c. of 1 per cent. (0.01 gram) anhydromethylenecitric acid was used in 10 c.c., 20 c.c. in some, and in others 2 c.c. of 1 per cent. (0.02 gram) in 20 c.c. were used. This will be indicated in Table 2, which contains data pertinent to the liberation of formaldehyd according to different degrees of alkalinity.

The results in Table 2 were confirmed in the following way: It will be recalled that phloroglucin reacts positively (producing a red color) with formaldehyd at a degree of alkalinity corresponding to p_H 12.13, but not at p_H 12.12. That is, the alkalinity required is at least that of a centinormal (N/100) sodium hydroxid solution. Mixtures of anhydromethylenecitric acid and sodium hydroxid were made up to correspond to the values of p_H 12.12, 12.13, 13.07 and 14.05 as in Table 2 and to each of these 0.5 c.c. of 1 per cent. phloroglucin in alcohol was added. The results were as follows: Yellowish color or negative for formaldehyd in the mixture with p_H values of 12.12 and 12.13; reddish or just positive in p_H 13.07, and deep red or strongly positive in p_H 14.05. The theoretical yield of formaldehyd in these mixtures was equivalent to a concentration of about 1 : 4,800, yet in the mixtures of p_H 12.13 in which the phloroglucin is readily positive with a concentration of 1 : 10,000 and even less, formaldehyd was not demonstrable by direct testing or by aeration.

Since it is known that a concentration of formaldehyd of 1 : 1,000,000 is readily recognizable by means of the phloroglucin test, the limit being about 1 : 10,000,000, it is obvious that whatever concentration might be present in the mixture of p_H 12.13 must be negligible for all practical purposes. The phloroglucin test with the mixture of p_H 13.07 was just about positive, indicating that this is about the lowest limit of alkalinity at which liberation of formaldehyd occurs. These tests were performed at room temperature. It is possible that a temperature equivalent to body heat might facilitate the decomposition so as to bring the limit of alkalinity below p_H 13.07.

TABLE 2.—LIBERATION OF FORMALDEHYD FROM ANHYDOMETHYLENECITRIC ACID ACCORDING TO DIFFERENT DEGREES OF ALKALINITY

pH Solutions Used and Quantity	Absolute Quantity of Anhydromethylenecitric Acid Used Gm.	pH of Final Mixtures	Free Formaldehyd (— = Absent + = Present)
18 c.c. phos.* = 8.4	0.02	7.4	—
18 c.c. N/10 $NaHCO_3$ = 8.2	0.02	8.2	—
2 c.c. N/10 NaOH to neutralize, then 20 c.c. N/10 NaOH=12.12	0.02	8.4	—
2 c.c. N/10 NaOH to neutralize, then 10 c.c. phos. = 8.4	0.02	8.4	—
2 c.c. N/10 NaOH to neutralize, then 10 c.c. N/100 NaOH = 12.13	0.02	12.13	—
2 c.c. N/10 NaOH to neutralize, then 10 c.c. N/10 NaOH = 13.07	0.02	13.07	+ (Equal to a concentration of formaldehyd of less than 1: 1,000,000 and more than 1: 10,000,000; just positive)
2 c.c. N/10 NaOH to neutralize, then 10 c.c. N/10 NaOH = 14.05	0.02	14.05	+ (Very strong)

* Phos. refers to mixtures of Na_2HPO_4 and NaH_2PO_4 made up to obtain pH values in the usual way.

Therefore, another set of tests was performed with mixtures of anhydromethylenecitric acid and sodium hydroxid ranging from p_H values of 12.12 to 14.05, as in Table 2. These were then incubated for fifteen to twenty minutes at 40 C. in a water bath, and then 0.5

c.c. of 1 per cent. phloroglucin in alcohol was applied to each tube. The limit for the positive test was precisely the same as when the same experiment was performed in the cold as described above. That is, formaldehyd was absent in the mixtures of p_H 12.12 and 12.13 and present in mixtures of p_H 13.07 and 14.05. The colors differed quantitatively only, being considerably deeper in the incubated tubes than in the cold solutions, indicating that the liberation of formaldehyd from, or, in other words, the decomposition of hydromethylenecitric acid is greatly accelerated with increase in temperature. Later it will be shown that longer heating at the boiling point decomposes almost all of it. Aside from this, the results thus far presented indicate that it is the degree of alkalinity, i.e., the hydroxyl ion concentration, which is of the greatest importance for the liberation of formaldehyd from anhydromethylenecitric acid.

It is, of course, highly improbable that such a degree of alkalinity as p_H 13.08, corresponding to the alkalinity of a decinormal (N/10) sodium hydroxid solution can ever be attained in the living organism. The following fluids must be considered in this connection, namely, pancreatic or intestinal juice, blood, urine and fermented or ammoniacal urine. There is plenty of evidence to indicate that intestinal juice is variable, being either very slightly alkaline [the alkalinity of N/70,000 K(OH) (Foa)[5]], very slightly acid or neutral [p_H 5.6 to 6.6 (McLendon, Shedlov and Karfan)[6]] in animals, and probably usually slightly alkaline in man. Since its alkalinity is due chiefly to sodium bicarbonate whose p_H value in high and low concentrations (N/10 to 2 to 3 per cent.) is about p_H 8.2 or 8.3, it is apparent that no formaldehyd can be liberated in the alimentary tract. The limit of alkalinity (p_H value) of the blood is about 7.4. The highest alkalinity attainable in urine after the administration of very large doses (40 grams) of sodium bicarbonate gives p_H values of 8.7 (Henderson and Palmer[7]).

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5. Foa: Soc. biol. **58:** 867, 1916; ibid. **58:** 1000, 1906.
6. McLendon, Shedlov and Karfan: J. Biol. Chem. **34:** 1, 1918.
7. Henderson and Palmer: J. Biol. Chem. **14:** 81, 1913.

This practically corresponds to the excretion of a solution of about a one-hundredth normal (N/100) disodium phosphate (Na_2HPO_4). Therefore, we may exclude intestinal juice, blood and urine (rendered alkaline in normal individuals) from the possibilities in which liberation of formaldehyd may occur. This leaves ammoniacal or fermenting urine in the bladder.

The almost total lack of data in the literature on the degrees of alkalinity attainable in fermenting urines leaves very little for consideration along this line. However, it is practically certain that no such degree of alkalinity as corresponds to that of a N/10 sodium hydroxid equal to the lowest p_H (13.07) necessary for liberation of formaldehyd is ever attained in the bladder. It is a well known fact that ammonia in the form of carbonate or hydroxid in aqueous solutions can not attain this degree of alkalinity. For instance, N/100 NH_4OH has the value of p_H 10.77 and N/1 NH_4OH of p_H 11.77 (L. Michaelis[8]), all of these representing concentrations of hydroxyl ions (alkalinity) less than necessary for the lowest limit (p_H 13.07) at which anhydromethylenecitric acid begins to liberate formaldehyd. Furthermore, bearing in mind the mutual avidity of these compounds, free formaldehyd can not exist in the presence of ammonia. Quantitative methods for estimating ammonia and formaldehyd based on this principle are well known, and I have shown in this paper that formaldehyd can be volatilized by aeration from as weak a solution as 1 : 1,000,000 and caught in dilute (0.4 per cent.) ammonia water.

These deductions and evidences are against anhydromethylenecitric acid occupying a serious position among urinary antiseptics. Even though it should be excreted unchanged in the urine, it is inconceivable that free formaldehyd would be liberated from it in the bladder. This was tested out and the evidences which were obtained fully confirm the deductions and conclusions reached above, even for highly ammoniacal urines. Before proceeding to this, it will be of interest to sub-

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8. Michaelis, L.: Wasserstoffionenkonzentration, 1914, Berlin.

mit the results obtained on the effects of acidity on hydromethylenecitric acid and its destruction in vitro.

Liberation of Formaldehyd from Anhydromethylenecitric Acid According to Different Degrees of Acidity (p_H).—This is of interest in connection with the liberation of formaldehyd in gastric juice and acid urine, although it is fairly well known that the decomposition of the compound does not proceed as readily in acid as in alkaline media. Mixtures of anhydromethylenecitric acid and hydrochloric acid of known hydrogen ion concentrations were made, aerated into ammonia water and the ammonia water was then tested with phloroglucin in alkali after appropriate treatment as described in the early part of the paper. The results were entirely negative for liberated formaldehyd in the following solutions with their p_H values of the final mixtures: N/100 HCl (p_H 3.4), N/10 HCl (p_H 1.2), N/1 HCl (p_H more than 0.1 and less than 1.0). The p_H values of the final mixtures were necessarily greater, indicating weaker acidity, than those of the original strengths of hydrochloric acid, since hydromethylenecitric acid is itself a rather weak acid, acting as a "buffer." It is plain from these data that no liberation of formaldehyd could occur in any of the acid fluids of the body, since these are much weaker than the concentrations of hydrochloric acid tried. When a solution of anhydromethylenecitric acid is layered on concentrated sulphuric acid containing phenol (Liebermann's test), a red color develops, indicating the presence of liberated formaldehyd.

Destruction of Anhydromethylenecitric Acid in Vitro.—Theoretically, the formaldehyd content of anhydromethylenecitric acid is about 14.7 per cent. This was substantiated by direct analysis. The formaldehyd was estimated quantitatively by the colorimetric method of Hanzlik and Collins[9] previously described. One cubic centimeter of 1 per cent. (0.01 gram) anhydromethylenecitric acid was made strongly alkaline with 1 c.c. of 20 per cent. sodium hydroxid and partly diluted with water. To this was added 1 c.c. of phloroglucin in alkali and the whole was diluted to 50 c.c. in a short Nessler tube; and compared with a series of standards of congo red and methyl orange representing different concentrations of formaldehyd as directed in the method. The solution matched a concentration of 1 : 35,000 or about 0.003 per cent., which on calculation is the equivalent of 0.0015 gram of absolute formaldehyd or about 102 per cent. of theory (0.00147 gram). Results varying from 98 to 102 per cent. were obtained.

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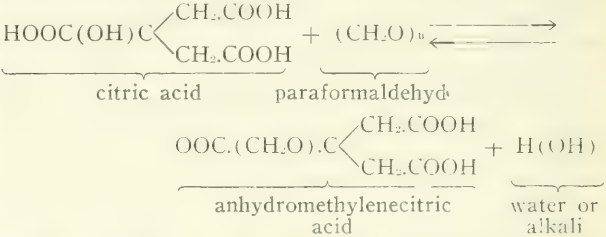
[9] Hanzlik and Collins: J. Biol. Chem. **25:** 231, 1916.

The results were quite different when 0.01 gram of anhydromethylenecitric acid was boiled and distilled with alkali. The formaldehyd content of the distillate was estimated also colorimetrically, but the recoveries in two different trials were 0.0006 gram and 0.00089 gram, or about 41 per cent. and 62 per cent. respectively. The residues left behind after distillation was completed were free from formaldehyd as indicated by negative tests with phloroglucin. These results indicate considerable destruction of the anhydromethylenecitric acid. What happens to the formaldehyd was not ascertained. It may be oxidized to formic acid or polymerized, being converted into the para form $(CH_2O)_n$ or formose $(CH_2O)_6$. Presumably it is oxidized, since treatment of formaldehyd solutions with alkalai hydroxids is known to transform formaldehyd into formic acid and methyl alcohol (Richter-Spielmann: Organic Chemistry, vol. i, 1919, p. 198), while distillation of aqueous solutions of formaldehyd or hexamethylenamin alone yields 98 to 100 per cent. recoveries.

Weak aqueous solutions of anhydromethylenecitric acid gradually liberated formaldehyd on standing. That is, decomposition of the acid is continuous and this is accelerated by heat and alkalies. The decomposition in water was repeatedly shown by simple aeration of standing solutions into ammoniated water and testing for formaldehyd in the usual way. Precaution was exercised, therefore, by using freshly prepared solutions in all experiments performed with the compound.

The longer the solutions stood the more formaldehyd was detectable. Incubation of aqueous solutions alone facilitated the liberation of formaldehyd. While even a high degree of acidity in the cold did not liberate formaldehyd, incubation at 37.5 C. with the same degrees of acidity (p_H 0.1 and 1.0) yielded formaldehyd definitely.

The decomposition with liberation of formaldehyd appears to be a reversible reaction, as illustrated by the following equation:



$$\underbrace{\mathrm{HOOC(OH)C}\Big\langle\begin{matrix}\mathrm{CH_2.COOH}\\ \mathrm{CH_2.COOH}\end{matrix}}_{\text{citric acid}} + \underbrace{\mathrm{(CH_2O)_n}}_{\text{paraformaldehyd}} \rightleftarrows \underbrace{\mathrm{OOC.(CH_2O).C}\Big\langle\begin{matrix}\mathrm{CH_2.COOH}\\ \mathrm{CH_2.COOH}\end{matrix}}_{\text{anhydromethylenecitric acid}} + \underbrace{\mathrm{H(OH)}}_{\text{water or alkali}}$$

Having ascertained sufficiently the behavior of anhydromethylenecitric acid physically and chemically, we may now proceed to the experiments on excretion. From what was observed in vitro, it was anticipated that these would be entirely negative so far as liberation of formaldehyd in urine is concerned. The quantitative excretion was also expected to be small. However, it was deemed necessary to supply actual data in this direction because of the claims that are made by Impens[1] for anhydromethylenecitric acid, and by the manufacturers for "helmitol."

Excretion of Anhydromethylenecitrate and Liberation of Formaldehyd in Urine.—Two experiments were performed. On two different occasions subjects P. J. H. took 1 gram of anhydromethylenecitric acid, once with and once without alkali. Urine was collected at end of fifteen minutes, one-half hour and at end of each hour therafter until the excretion ceased as indicated by the phloroglucin test applied to a specimen of urine previously raised to the boiling point with 5 to 10 per cent.

sodium hydroxid. Each specimen of urine was carefully measured, and the excretion of the citrate determined quantitatively by the colorimetric method for formaldehyd previously described by Hanzlik and Collins.[9] The hydrogen ion concentrations (p_H values) of the urines were also determined colorimetrically in the usual way. The urines were analyzed immediately after voiding. They were also aerated for free formaldehyd as described in the fore part of the paper. The results of the two experiments are presented in the following protocols.

EXPERIMENT 1.—AT 12:05 A. M.

Subject P. J. H. took by mouth 1 gm. anhydromethylenecitric acid

Number of Urine Specimen	Period (End of) Hours	Volume Voided, C.c.	Reaction to Litmus	Anhydromethylenecitric Acid (+ = Present)	Quantity of Anhydromethylenecitric Acid Excreted, Expressed as Gram HCHO	Free Formaldehyd (by Aeration) (— = None)
1	¼	30	Acid	+ (weak)	0.00003	—
2	½	65	Acid	+ (definite)	0.00033	—
3	1	290	Acid	+ (strong)	0.00235	—
4	2	240	Acid	+ (strong)	0.00240	—
5	3	50	Acid	+ (less strong)	0.00063	—
6	4	30	Acid	+ (weak)	0.00038	—
7	5	40	Acid	– (?)	0.00003	—
Total excreted					0.0064 or 4.4 %	

It is seen that the excretion begins rather promptly, the compound being detectable in urine in fifteen minutes after administration. This was anticipated because of its fairly good solubility in alkalies as indicated in the fore part of the paper. The excretion appears to reach its maximum at the end of about one hour and is practically completed at the end of five to six hours. This, of course, would depend on the diuresis, which in these experiments was maintained at a fairly high level by liberal ingestion of water. Free formaldehyd was absent in all specimens of both experiments, even in the alkaline series (Experiment 2). This would be expected, since the alkalinity was not high enough for liberation of formaldehyd. It will be recalled that an alkalinity of about p_H 13.07 is necessary for this.

There is no doubt that anhydromethylenecitric acid is present as such in the urine. Direct application of the phloroglucin test in alkali without previous heating gave, as a rule, practically no color, except with the very concentrated urines. However, when the urines were previously raised to the boiling point with a little 20 per cent. alkali and then the phloroglucin test was applied, there was an immediate development of red color, this being very deep with the more concentrated urines. This means that the formaldehyd is in combined form, in other words, as anhydromethylenecitrate, which requires decomposition by alkali as in pure aqueous solution before the formaldehyd is liberated. As indicated by the results of both experiments, the quantity of anhydromethylenecitrate excreted is very small (4.4 and 4.6 per cent.), indicating that the compound is almost entirely destroyed in its passage through the body. The feces were not examined, but its solubility and rapid excretion indicate that it is completely absorbed just as hexamethylenamin is. It was previously observed with similar dosage that about 80 per cent. of hexamethylenamin is excreted in urine. No disturbances of the alimentary tract such as nausea, vomiting and diarrhea were experienced after anhydromethylenecitric acid.

EXPERIMENT 2

One hour previously Subject P. J. H. took by mouth 10 grams sodium bicarbonate; at 10:35 a. m., took 1 gram anhydromethylenecitric acid, and ont hour after this, 5 grams of bicarbonate to maintain urine alkaline

Number of Urine Specimen	Period (End of) Hours	Volume Voided, C.c.	Reaction P_H	Anhydromethylenecitric Acid (+ = Present)	Quantity of Anhydromethylenecitric Acid Excreted, Expressed as Gram HCHO	Free Formaldehyd (by Aeration) (— = None)
1	¼	255	7.9	+ (weak)	....	—
2	½	210	7.6	+ (definite)	0.00053	—
3	1	290	7.4	+ (strong)	0.00300	—
4	2	320	7.7	+ (strong)	0.00160	—
5	3	100	7.9	+ (strong)	0.00050	—
6	4	80	8.0	+ (less strong)	0.00080	—
7	5	50	7.9	+	0.00063	—
8	6	40	7.8	+ (?)	....	—
Total excreted					0.00710 or 4.6 %	

As to the nature of the destruction it is suggested that the citric acid portion of the molecule is oxidized just as ordinary citric acid is, a portion of the compound escaping because of the rapid excretion. The formaldehyd portion is presumably oxidized to formic acid as usual. There are no data for deciding whether the oxidation of the formaldehyd radical occurs before or after that of the citric radical.

The results on excretion are confirmative of the tendency of the behavior of anhydromethylenecitric acid in vitro. It was found to be quite readily destroyed with moderately prolonged heating and alkali and distillation. The results indicate that the compound could not be excreted in sufficient concentration to impart especially valuable antiseptic qualities to urine. It certainly could not surpass hexamethylenamin, even for alkaline urines, whose alkalinity can be altered by means of diuresis and administration of acids or acid salts. Greater difficulties are, of course, encountered with the antisepsis of strongly ammoniacal or decomposing urines, as in cystitis. It is alleged that "helmitol" (hexamethylenamin anhydromethylenecitrate) is superior to hexamethylenamin in this condition. The advantage, it is claimed, depends on the property of "helmitol" liberating formaldehyd from its anhydromethylenecitrate component in alkaline media. A priori this proposition can be safely answered in the negative on the basis of the results, and deductions drawn therefrom, in the fore part of the paper. Nevertheless, some observations were made along this line.

Liberation of Formaldehyd from Anhydromethylenecitrate in Ammoniacal Urines.—A portion of each urine of Experiments 1 and 2 was allowed to stand at room temperature, and another portion was incubated at 38 C. for the development of ammonia. This occurred in all specimens on the day following the administration of the drug, i.e., in about twenty hours. The urines were all precipitated and foul smelling. They were allowed to stand for several days and aerated into ammonia water and tested with phloroglucin as described previously for the liberation of free formaldehyd. The true reactions were obtained colorimetrically by matching against standards of definite p_H values in the usual way. The results are presented in Table 3.

It is seen that the presence of free formaldehyd could not be demonstrated in even the most ammoniacal urine (p_H 12.12). Bearing in mind that anhydromethylenecitric acid or the citrate, does not begin to liberate formaldehyd until the alkalinity corresponding to p_H 13.07 is reached, it is obvious why formaldehyd could not be demonstrated in these urines. That is, none was sufficiently alkaline, the highest alkalinity reached being the equivalent of p_H 12.12. The urines standing at room temperature were even less alkaline as indicated by the p_H values of 7.1 to 8.4. Therefore, no liberation would be expected in these, and no formaldehyd was found. The highest alkalinities observed ($p_H = 12.12$) corresponded to the alkalinity of a solu-

TABLE 3.—LIBERATION OF FORMALDEHYD FROM ANHYDROMETHYLENECITRATE IN AND REACTION OF AMMONIACAL URINES

Urines of Experiment 1				Urines of Experiment 2			
Number of Specimen	Ammoniacal and Foul (+ = Positive)	P_H Value End of 72 Hours	Free Formaldehyd by Aeration (— = Absent)	Number of Specimen	Ammoniacal and Foul (+ = Positive)	P_H Value End of 72 Hours	Free Formaldehyd by Aeration (— = Absent)
			At Room Temperature				
1	+	.....	—	1	+	.....	—
2	+	12.12	—	2	+	12.12	—
3	+	7.3	—	3	+	12.12	—
4	+	8.2	—	4	+	8.4	—
5	+	7.1	—	5	+	8.0	—
6	+	7.2	—	6	+	8.0	
				7	+	.....	—
			Incubation at 38 C.				
1	+	12.0 (about)	—	1	+	.....	
2	+	12.12	—	2	+	12.12	
3	+	8.0	—	3	+	12.12	—
4	+	<12.2	—	4	+	12.13	—
5	+	....		5	+	12.0 (about)	
6	+	.....	—	6	+	.....	—
				7	+	.....	—

tion of N/100 sodium hydroxid, or what is equally probable, that of normal (N/1) ammonium hydroxid whose p_H value = 11.77. In urines such differences as occur between the values of $p_H = 12.12$ and $p_H = 11.77$ are practically negligible as it is not always easy to match the colors precisely because of the interference from color of urine itself. In these particular urines the turbidity also interfered somewhat and was difficult to remove by filtration. We may say, therefore, that the ammoniacal alkalinity of these decomposed urines corresponded closely to that of a normal (N/1) solution of ammonium hydroxid, or 1.7 per cent. of ammonia.

It is reasonably certain that such a degree of alkalinity is never met with in life, and indeed would be practically intolerable to the bladder mucosa. Continuous excretion of urine would have the tendency to maintain the ammoniacal decomposition, therefore the alkalinity, at a lower level. Granted that the alkalinity necessary for the liberation of formaldehyd from anhydromethylenecitrate might be reached by ammonia, the liberated formaldehyd would be at once converted into hexamethylenamin because of its great avidity for the ammonia. This principle is used for the quantitative estimation of formaldehyd or ammonia as the case may be. This would leave a compound not decomposable in alkaline media, bringing us back to the more rational method of therapeutic treatment, namely, rendering the urine acid first and then administering hexamethylenamin in the usual way. Needless to say, none of the urines containing anhydromethylenecitrate remained sterile, as indicated by the prompt ammoniacal decomposition, even those rendered previously alkaline (Experiment 2).

From all those, it is to be concluded that, as a urinary antiseptic for acid, alkaline and ammoniacal urines, anhydromethylenecitric acid is a complete failure. It has been adequately shown by the results of this study that formaldehyd liberation from this compound does not and can not occur under conditions of chemical reaction in the body. This is the chief reason for its failure as an antiseptic. Its antiseptic efficiency would be weakened further because it is almost entirely destroyed in its passage through the body.

"Citarin."—This is the sodium salt of anhydromethylenecitric acid. Since the acid is neutralized by the bicarbonate of the intestine, what is absorbed into the circulation and excreted into urine is really the sodium salt. Therefore, what has been said throughout the text about anhydromethylenecitric acid as to destruction, liberation of formaldehyd and excretion applies to "citarin." Hence, its efficiency as a urinary antiseptic is to be regarded as negligible.

"Helmitol."—This is said to be hexamethylenamin anhydromethylenecitrate. The behavior of its anhydromethylenecitric acid component is strictly the same as that of the pure acid, except for some difference in acidity. Quantitatively, its relations would be about one-half those of the pure acid. The mechanism of action of hexamethylenamin is well understood from work previously reported.[10] This portion of "helmitol," of course, would act no differently from hexamethylenamin. "Helmitol," therefore, represents nothing original over its components as a contribution to the field of urinary antisepsis. At any rate it can claim no special usefulness because of its anhydromethylenecitrate component.

SUMMARY

1. Anhydromethylenecitric acid is slowly soluble in water and acids and more readily in alkalies, which decompose it with the liberation of free formaldehyd.

2. The degree of alkalinity necessary for this is rather high as indicated by the hydrogen ion concentration, requiring a *p*H value of 13.06, corresponding to the alkalinity of a decinormal sodium hydroxid solution. High concentrations of bicarbonate (N/10, 1 and 2 per cent.) do not liberate formaldehyd from anhydromethylenecitric acid.

3. Very high degrees of acidity (concentrated sulphuric acid) also decompose anhydromethylenecitric

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[10] Hanzlik and Collins: Arch. Int. Med. **12**: 578, 1913.

acid. On incubation, a lower acidity suffices, namely, $p_H = 0.1$ and $p_H = 1.0$.

4. Decomposition by hydrolysis with liberation of formaldehyd occurs in aqueous solutions on standing.

5. Moderate heating and distillation with alkali destroy about one-half of the anhydromethylenecitric acid.

6. The decomposition with formaldehyd liberation appears to be a reversible reaction.

7. Anhydromethylenecitric acid is excreted as the citrate in urine to the extent of only about 4.5 per cent., the remainder being probably destroyed in its passage through the body.

8. Free formaldehyd is not demonstrable in either acid, alkaline or highly ammoniacal urines containing anhydromethylenecitrate. This would be expected, since the alkalinity attained (p_H 11.77 to 12.12) even in the most ammoniacal urines was not that required (p_H 13.07) for the liberation of formaldehyd. It is doubted whether this degree of alkalinity is attainable during life.

9. The administration of anhydromethylenecitric acid does not confer antiseptic qualities on urines previously rendered alkaline by the administration of bicarbonate, and even on ammoniacal urines. All such urines decompose readily.

10. "Citarin," or sodium anhydromethylenecitrate, behaves precisely the same way as anhydromethylenecitric acid after administration, and neutralization by the bicarbonate of the alimentary tract. Its antiseptic qualities are to be regarded as negligible.

11. "Helmitol," or hexamethylenamin anhydromethylenecitrate, can behave no differently than its components. Except for its action as a hexamethylenamin compound, it is inert and negligible as an antiseptic.

12. The rational use of hexamethylenamin as a urinary antiseptic still remains unsurpassed among this class of agents.

IODIN ABSORPTION FROM THE HUMAN SKIN *

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AND
TORALD SOLLMANN
CLEVELAND

(From The Journal of Pharmacology and Experimental Therapeutics, April, 1920, p. 169)

The impression prevails widely that free iodin is absorbed quite freely from the skin. For instance, Heffler [1] states:

> After application of iodin to the most various parts of the body, or after inhalation of the vapor, it appears in the urine in the form of iodids. More exact data are wanting as to the time between application and appearance in the urine; except that with reference to the epidermal application of iodin tincture. V. Rozsahegyi states that iodin is demonstrable in the urine after two and one-half hours. In general, it is believed that iodin is excreted more slowly from all places of application except the skin, than are the alkaline iodids.

Von Jaksch [2] writes:

> . . . in view of the facility, with which iodin in every form is absorbed by all organs of the body and then appears in the secreta and excreta, this also after the application of iodin preparations to the skin and mucous membranes . . .

However, we have only encountered a single reference to actual observations on this subject, namely, that by Heffler to Rozsahegyi. The original is not accessible to us.

The following observations indicate that the normal human skin is a very poor channel for the absorption of iodin, even in the free state. The experiments were carried out by N. C. Wetzel.

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* From the Department of Pharmacology of the Medical School, Western Reserve University, Cleveland.

* Received for publication Jan. 20, 1920.

* This investigation was supported by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.

[1] Heffler, A.: Ergeb. Physiol., 1902, p. 104.

[2] v. Jaksch, R.: Die Vergiftungen, 1897, p. 97.

Method.—Iodin was sought by the usual method: viz., the addition of dilute sulphuric acid and sodium nitrite, and shaking out with chloroform. This acquires a pink color in the presence of iodin. The test was applied directly to 15 to 25 c.c. of the urines collected at various periods. Larger samples of the mixed urine of twenty-four hours (200 c.c. representing about one-eighth of the daily urine) were evaporated and incinerated with potassium nitrate and sodium carbonate dissolved in water, and acidified with sulphuric acid before the test was applied.

Delicacy of Test.—Experiment 1.—Sodium iodid was added to a urine in the ratio of 0.0117 per cent., equivalent to 0.01 per cent. of iodin. Definite quantities of this were tested; with and without incineration. The results were the same in both cases.

Eight-hundredths (0.08) c.c. of the urine, representing 0.008 mgm. of iodin, still gave a faint but quite definite pink. This may be considered as the limit.

Experiment 2.—In Test 1 only a trace of urine was present. In order to approximate the actual condition, definite quantities of a watery iodid, 1 c.c. = 0.1 mgm. iodin, were added to larger quantities of urine (60 to 200 c.c.). These were then tested with and without incineration. Without incineration a good pink color was obtained with 0.01 mgm. of iodin. This agrees with the statement of Sandland[3] that 0.05 mgm. of KI can be demonstrable in 5 c.c. of urine. After incineration the results were uncertain at 0.05 mgm., but quite positive at 0.1 mgm. The limit of sensitiveness of the method, as applied to urine, may therefore be stated as: 0.01 mgm. of iodin, when applied directly; 0.1 mgm. of iodin, when applied after incineration.

Application of Iodin.—The following preparations of iodin were applied to the normal human skin. The tinctures were painted on the palmar surface of the forearm. The other preparations were rubbed thoroughly into the skin of the chest and abdomen.

Preparations of Iodin.—Experiment 3.—Tincture of iodin, U. S. P., 0.1 c.c., representing 7 mgm. of iodin and 5 mgm. of potassium iodid.

Experiment 4.—The same, 0.5 c.c., representing 35 mgm. of iodin and 25 mgm. of potassium iodid.

Experiment 5.—Same as Experiment 4.

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[3] Sandland, H.: Quoted from Neubauer and Vogel, Analyse des Harns, 1898, p. 606.

Experiment 6.—Iodin ointment, U. S. P., 5 grams, representing 200 mgm. of iodin and 200 mgm. of potassium iodid.

Experiment 7.—"Iodex," 5 grams. This is a proprietary iodin ointment, for which absorption by the skin has been claimed. It was claimed to contain 5 per cent. of free iodin, but according to analyses by the Chemical Laboratory of the American Medical Association,[4] it contains only minute traces of free iodin, and 2.63 per cent. of total iodin, appearing in combination with petrolatum. The 5 grams would therefore represent 132 mgm. of total iodin.

Experiment 8.—Iodized poppyseed oil ("Iodoleine—Dubois"), 5 grams containing 25 per cent. (1.3 gram) of combined iodin.

Collection of Urine and Results.—In each experiment, the total urine was collected in convenient fractions during twenty-four hours. Samples of these urines were then tested directly. The remainder of the specimens was united, and 200 c.c. of the mixture, which represented about one eighth of the total daily urine, was tested after incineration. In view of the prompt excretion of iodin, the collection was not continued after twenty-four hours.

Results.—None of the experiments revealed a trace of iodin. The total quantity of iodin excreted was therefor less than 1.6 mgm. and may have been zero.

DISCUSSION

The failure to excrete iodin does not necessarily mean that none has been absorbed; for if only very small amounts have been absorbed, it is conceivable that they may have been retained completely in the body. However, it is clear that the absorption of iodin through the skin is not nearly as extensive as is commonly supposed.

It may also be pointed out that the results refer to single applications to normal skin. It is quite probable that if the skin is injured, for instance by repeated applications of strong iodin solution, its permeability may be increased. This would doubtless occur if there has been actual vesication, or probably even if the epidermis has been desquamated.

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[4] Reports of the Council on Pharmacy and Chemistry of the American Medical Association, 1915, p. 144.

CONCLUSIONS

The urine does not contain demonstrable quantities of iodin compounds after the application of iodin tincture, or the official or proprietary ointments, to the normal human skin.

The amount of iodin absorbed by the normal human skin must therefore be very small.

It is probable that larger quantities would be absorbed if the skin were injured by the repeated application of strong iodin preparations.

TYRAMINE AS A MORPHIN ANTAGONIST *

HENRY G. BARBOUR

AND

LLOYD L. MAURER

NEW HAVEN

(From The Journal of Pharmacology and Experimental Therapeutics, June, 1920, p. 305)

INTRODUCTION

The prime requisite of an ideal morphin antagonist is respiratory stimulation without removal of analgesia. Probably the two most important indications for its employment would be, firstly, as a physiologic antidote in poisoning, and secondly, as a preventative of morphin asphyxia in the new-born, widening the obstetrical use of morphin.[1] The work herein reported was done with particular reference to the latter case, which would presumably call for employment of the two drugs simultaneously. Tyramin attracted our attention as a possible respiratory stimulant when Bry [2] pointed out its prolonged effect on the respiration rate in cats.

The respiratory stimulants commonly employed to antagonize morphin depression (caffein, atropin, scopolamine, narcotin, etc.), do not possess the advantage of ecbolic action which is exhibited by tyramin.

Tyramin (para-oxyphenylethylamin) has been known since the work of Barger and Dale [3] as the most powerful pressor of ergot. The occurrence of the amin in this fungus as well as in putrid meat, cheese, etc., is

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* From the Department of Pharmacology of the Yale University School of Medicine.

* Received for publication April 17, 1920.

* This investigation was supported by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and from the Francis E. Loomis Research Fund of the Yale University School of Medicine. Some of the results were referred to in a preliminary communication: Barbour, H. G., Maurer, L. L., and von Glahn, W. C., J. Pharmacol. & Exp. Therap., **8**: 124, 1916.

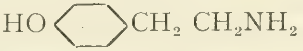
[1] Marbour, H. G.: J. A. M. A. **69**: 882, 1917.

[2] Bry, G.: Ztschr. f. Exper. Path. u. Therap. **16**: 186, 1914.

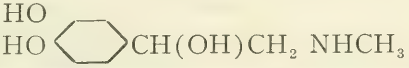
[3] Barger, G., and Dale, H. H.: J. Physiol. **41**: 19, 1910.

attributed to its origin by the splitting off of CO_2 from tyrosin (Barger and Walpole [4]). The best known actions of tyramin are "sympathomimetic" for it bears a close chemical relationship to epinephrin.

tyramin



epinephrin



Outside of the present connection its employment in therapy appears to have been limited to circulatory stimulation, in which respect it has been particularly studied by Hewlett.[5]

In the preliminary work on tyramin as a morphin antagonist, which forms the subject of this paper, it was necessary to employ small animals on account of the scarcity of the drug at that time; We therefore chose rats. An apparatus was developed for respiratory studies in these animals and the scope of the investigations included the following: the effects upon the respiratory volume of morphin and of tyramin separately and combined, the optimum doses for antagonism, the rôle of absorption, and the relation of the antagonism to morphin analgesia. Studies were also made of carbon dioxid (7 to 8 per cent.) breathing with relation to the various doses. This respiratory stimulant, although known since the time of Loewy [6] to be ineffectual against morphin alone, was employed to throw further light on the condition of the respiratory mechanism throughout the experiments as well as to test the possibility of a synergic action with tyramin. The commercial preparation known as "Tyramin-Roche" was used throughout the work.

METHOD

Apparatus.—The apparatus employed (Fig. 1) consisted of three parts; a wire mesh jacket for the animal, a head-piece and a pair of Müller water valves.

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4. Barger, G., and Walpole, G. S.: J. Physiol. **38:** 343, 1909.
5. Hewlett, A. W., and Kay, W. E.: J. A. M. A. **70:** 1810, 1918.
6. Loewy: Arch. f. d. ges. Physiol. **47:** 601, 1890.

Soft wire mesh cylinders of differing sizes were constructed to fit snugly animals of various weights. A small dorsal window was provided for the subcutaneous injection of drugs. Leather straps with buckles served to close the ends of the cylinder. A hole was made in the anterior strap for the neck of the head piece, a smaller one in the posterior strap for the tail of the rat. The animal could thus be securely held in an upright position with ample room for respiratory movements without the use of an anesthetic. The wire netting provided for free skin ventilation.

The head piece was made from the top of a wide-mouthed glass bottle cut off about 4 cm. from the neck. It was of such a size as to slip easily into the end of the wire cylinder, a little modeling clay assisting in making it conform in shape to the head of the rat. Rubber dam was tied tightly over the bottom of the head piece, a 1 cm. hole in the center of this diaphragm sufficing to admit the animal's snout. A perforated rubber stopper in which a large cone-shaped depression accommodated the nostrils served to hold the glass T-piece connecting with the water valves. The leg of the T-tube was cut so short as practically to eliminate any artificial dead space.

From wide mouthed bottles of about 50 c.c. capacity were made suitable Müller valves. The outlet valve was connected either directly with a 1-liter Danish gas meter or with a very light rubber bag, whence the expired air after collection could be expressed through the meter. By means of a T-tube on the inlet side of the apparatus a change could be made at any time from room air to that containing a known percentage of carbon dioxid. Tests of the apparatus showed that normal animals could remain in it over a period of seven to eight hours without exhibiting significant variations in the rate or volume of the respiration.

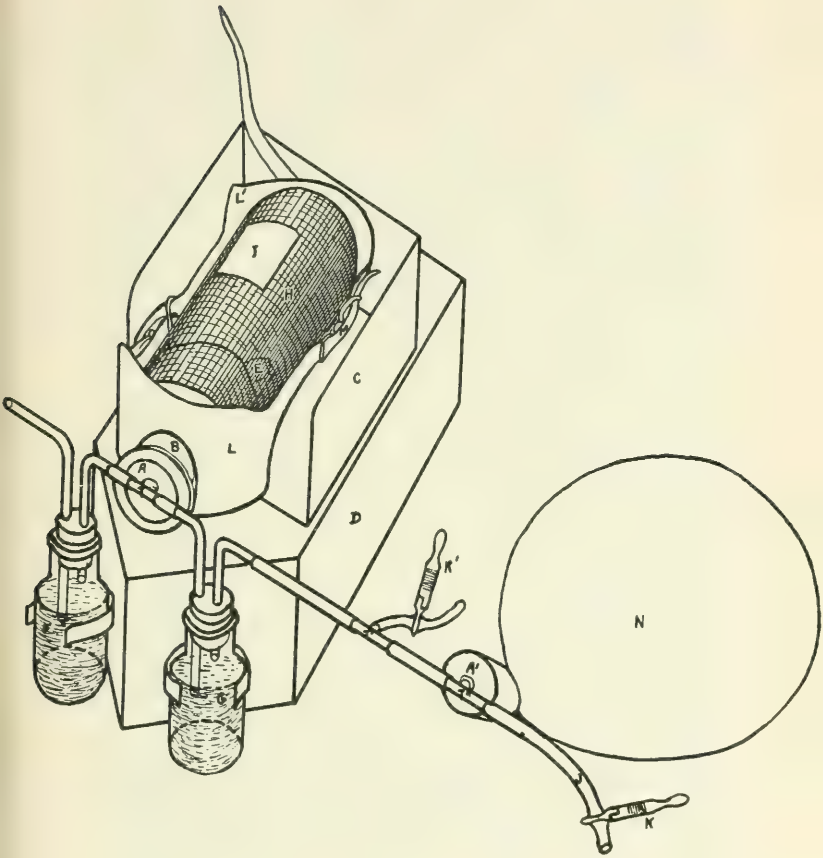


Fig. 1.—Respiratory Apparatus for Rat.—The dimensions given are suited to an animal weighing 150 grams. *A, A'*, rubber stoppers. The inner surface (not seen) of *A* has been cut to fit nose of rat; *B*, neck (diameter 2.2 cm.) of bottomless bottle (diameter 3.8 cm., length 5 cm.) padded with modeling clay to fit head of rat; *C*, cardboard tray; *D*, wooden box; *E*, rat's left ear, posterior to bottle edge which is fitted with rubber dam (not seen). *F*, inflow; *G*, outflow, Müller water valve (bottle 5 by 2.5 cm.); *H*, soft wire mesh cylinder (10 by 4.8 cm.); *I*, injection window in same; *J*, rubber tubing leading to meter *K*, *K'*, clamps; *L*, *L'*, anterior and posterior leather straps with holes for bottle neck and rat's tail, respectively; *M*, left buckle; *N*, light rubber bag; *T*, *T'*, *T"*, glass T-tubes (inside diameter 3 mm.).

Procedure. — In each experiment the rat was weighed and after the hair about its nostrils had been cut close, it was coaxed into the apparatus. Ten or fifteen minutes were always allowed for adjustment of the animal to its new surroundings, the expired air during three minute periods at frequent intervals was collected and measured, the respiratory rate being determined simultaneously. The carbon dioxid periods, in all experiments where this gas was breathed, were always preceded immediately by the air breathing periods and were of the same duration as the latter.

With scarcely any exceptions, a new rat was used for each experiment, so that the factor of morphin tolerance was eliminated. Each animal was fasted at least twenty-four hours before experimentation. The solutions were prepared freshly each day and all injections made subcutaneously. Fresh carbon dioxid air mixtures were made and the percentage determined by analysis (Haldane) each day. The bag which contained the mixture was frequently tested for leakage.

The normal average respiratory volume and rate were estimated by two or more determinations, the carbon dioxid determinations being made under the same conditions. The injection of the drug or drugs was made a few minutes subsequent to the last of these, after which respiratory volume and rate were followed at half-hourly or shorter intervals until the maximum effects of the injection had been attained. Observations were then made at less frequent intervals, usually until conditions had returned to normal. This procedure will be clear from Protocol I.

RESULTS

Effects of CO_2 and Morphin Separately and Together.—The effects of breathing 7 to 8 per cent. carbon dioxid and of adequate doses of morphin separately and together may all be illustrated by reference to the protocol of a typical experiment. (See Protocol I.)

It will be seen that a medium dose of morphin given to a rat whose average normal respiratory volume was 138 c.c. and rate 172 per minute, reduced these figures to a minimum of 60 c.c. and 88 respectively, making the volume 43 per cent. and the rate 51 per cent. of the normal. It will be further seen that carbon dioxid breathing under the same conditions increased the average respiratory volume to 195 c.c. (140 per cent. of normal), the rate being slightly decreased in this case. After morphin, however, carbon dioxid breathing was able to elicit but little response; the respiratory volume under CO_2 and morphin together attained only

PROTOCOL I.—AUGUST 3, 1915. RAT A5, (175 GRAMS).
PERCENTAGE OF CO_2, 7.6

		Air Breathing		Air + CO_2 Breathing	
Time	Meter	Expired per Minute, C.c.	Rate per Minute	Expired per Minute, C.c.	Rate per Minute
10:30 a. m.	0.35	...	...	...	...
10:33 a. m.	0.785	145	188	...	...
10:36 a. m.	1.33	...	...	182	188
11:00 a. m.	0.28	...	...	...	...
11:03 a. m.	0.64	129	168	...	...
11:06 a. m.	1.24	...	...	200	148
11:30 a. m.	0.88	...	...	...	...
11:33 a. m.	1.33	150	160	...	...
11:36 a. m.	1.94	...	...	203	152
11:40 a. m.	Injection 2 mg. morphin sulphate subcutaneously (1.14 mg. per 100 gm. rat)				
12:00 m.	0.865	...	...	...	...
12:03 p. m.	1.05	62	88	...	..
12:06 p. m.	1.25	...	...	67	100
12:30 p. m.	0.825	...	...	...	...
12:33 p. m.	1.005	60	92	...	...
12:36 p. m.	1.215	...	...	70	..
2:30 p. m.	0.47	...	...	...	...
2:33 p. m.	0.75	93	128	...	...
2:36 p. m.	1.08	...	...	110	140
3:30 p. m.	0.50	...	...	...	...
3:33 p. m.	0.83	110	140	...	...
3:36 p. m.	1.20	...	...	123	136
4:30 p. m.	0.35	...	...	...	...
4:33 p. m.	0.76	137	176	...	...
4:36 p. m.	1.36	...	...	200	184
Normal average		138	172	...	...
CO_2 average		...	...	195	163
CO_2 per cent. of normal		...	...	140	95
Morphin:					
Minimum		60	88	...	...
Per cent. of normal		43	51	...	...
Morphin + CO_2					
Minimum		...	...	67	100
Per cent. of normal		...	...	49	58

49 per cent. of normal at the minimum point (rate, 58 per cent.). In Figure 2 is shown graphically the details of another typical experiment (Rat 6, 180 grams).

The results of eleven such morphin experiments are summarized in Table 1. In eight of these parallel determinations of the effects of CO_2 breathing were made.

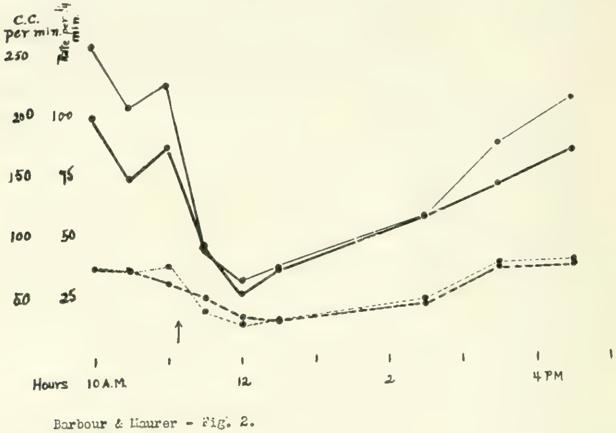


Fig. 2 (Rat 6, 180 grams).—Injection (at arrow) of morphin sulphate, 1.11 mgm. (per 100 grams). Heavy lines, air breathing; light lines, carbon dioxid breathing; continuous lines, respiratory volume, cubic centimeter per minute; broken lines, respiratory rate per quarter minute.

From the fourth column it will be seen that the doses of morphin given ranged from 0.5 mg. per 100 grams rat. In the next column is shown the normal average respiratory volume per minute of each rat; the increases in eight rats under 7 to 8 per cent. CO_2 breathing are shown in column 6. The average of column 7 reveals the fact that the concentration of carbon dioxid employed increases the respiratory volume by about 36 per cent.

The average minimum respiratory volume under morphin was 54 c.c. per minute or 40 per cent. of normal (columns 8 and 9), while the minimum under CO_2

breathing in the morphinized rats averaged 64 c.c. per minute or 43 per cent. of the normal of the same rats.

The respiratory rates were followed throughout every experiment and the final averages are appended at the end of the table. From these it will be seen that the rate was considerably reduced by morphin and that the carbon dioxid breathing gave practically no increase, 33 per minute to 34. The increased volume under CO_2 was therefore due to deepening of the individual respirations. Likewise in the morphinized rat CO_2 exerted no significant effect upon the number of respirations.

TABLE 1.—EFFECTS OF CO_2 AND MORPHIN, SEPARATELY AND TOGETHER

	Rat			Respiration, C.c. per Minute						
					7 to 8 per Cent. CO_2		Morphin		Morphin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Morphin Sulphate, Mg. per 100 Gm.	Normal Average	Average	Per Cent. of Normal	Minimum	Per Cent. of Normal	Minimum	Per Cent. of Normal
10/11	B3	120	0.50	112	...	...	40	36	..	..
8/ 3	5	215	0.93	167	233	139	73	43	80	48
9/21	8	170	1.00	130	...	...	67	52	..	..
11/12	B18	120	1.00	74	...	...	21	28	..	..
8/ 4	6	180	1.11	173	233	135	55	31	65	37
8/13	A7	135	1.11	147	198	135	70	48	75	51
8/ 3	A5	175	1.14	138	195	140	60	43	67	40
7/22	3	190	1.50	127	157	124	63	50	70	55
7/19	1	185	1.60	143	180	126	43	30	55	38
7/21	2	170	1.80	120	160	133	50	42	47	39
7/23	4	150	2.00	143	217	152	50	35	52	36
Average respiratory volume.....................				134	196	136	54	40	64	43
Average number respirations per ¼ minute*....				33	34	...	18	..	19	..

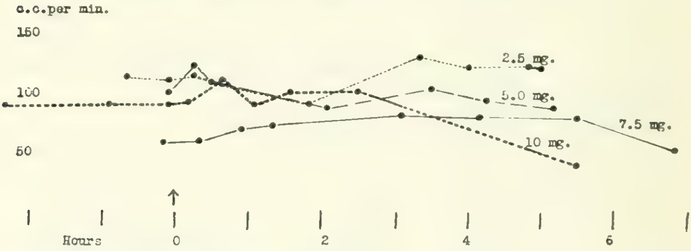
* Simultaneous with volume determinations in each experiment.

Effects of Tyramin.—In a similar manner the effects of tyramin (from 1.7 to 20 mgm. per 100 grams rat) were studied in seven different animals. The effects of tyramin upon the respiratory volume are illustrated in Tables 2 (1 to 10 mgm.) and 3 (15 and 20 mgm.). It will be seen that the smaller doses cause in five experiments an average increase of the respiratory volume to 124 per cent. of normal, the rate also being increased from an average normal of 26 to an average maximum of 32 per minute. To show the somewhat prolonged character of the moderate increases in respiratory volume, the volume curves of the five experiments are plotted in Figure 3.

The most satisfactory curve of prolonged respiratory stimulation was given by Rat B26, to which 7.5 mgm.

TABLE 2.—EFFECTS OF TYRAMIN (1 TO 10 MG. PER 100 GM. RAT)

	Rat			Respiration, C.c. per Minute						
					7 to 8 per Cent. CO_2		Tyramin		Tyramin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Tyramin—HCl, Mg. per 100 Gm.	Normal Average	Average	Per Cent. of Normal	Maximum	Per Cent. of Normal	Maximum	Per Cent. of Normal
7/20	A1	180	1.7	152	200	132	173	113	220	145
12/ 1	B24	120	2.5	115	...	...	132	115	...	...
12/ 1	B25	130	5.0	104	...	...	127	122	...	...
12/ 3	B26	120	7.5	61	...	...	87	142	...	...
11/ 3	B12	150	10.0	94	...	...	118	126	...	...
Average respiratory volume				105	...	..	127	124	...	...
Average number respirations per ¼ minute				26	...	...	32	...	...	...



Barbour & Maurer – Fig 3

Fig. 3.—Effect on respiratory volume of different rats of various doses of Tyramin. Light dotted line, rat B24, 2.5 mgm. (per 100 grams); light dash line, rat B25, 5 mgm. per 100 gm.; light continuous line, rat B 26, 7.5 mgm. per 100 grams; heavy broken line, rat B 12, 10 mgm. per 100 grams. All injections made at zero hour.

per 100 grams were given. (The maximum respiratory volume increases with each dose up to this point, declining again with larger doses.) In less complete experiments (not described herein) similar results have been obtained with this dose.

At the end of about an hour there is seen in the other curves a return to normal, followed sooner or later by a more prolonged volume increase. In the single experiment in which tyramin alone was combined with carbon dioxid breathing (Rat A1) the greatest stimulation of the respiratory volume in our entire series of experiments was noted, namely an increase to 145 per cent. of normal. As the increase produced by tyramin alone in this experiment amounted to 13 per cent. and that produced by carbon dioxid breathing alone to 32 per cent. it is interesting to note an exact summation of the effects of the two stimulants.

TABLE 3.—EFFECTS OF TYRAMIN (15 TO 20 MG. PER 100 GM. RAT)

				Respiration, C.c. per Minute				
					Tyramin			
Date	Rat Number	Weight, Gm.	Tyramin—HCl, Mg. per 100 Gm.	Normal Average	Maximum After Drug	Per Cent. of Normal	Minimum	Per Cent. of Normal
12/3	B27	140	15	93	81	87	37*	39
11/3	B11	130	20	161	163	101	30†	18
Average respiratory volume........				127	122	94	34	29
Average number respirations per one-quarter minute................				23	26	..	12	..

* Five and one-half hours after injection experiment discontinued.

† Six and three-quarter hours after injection experiment discontinued.

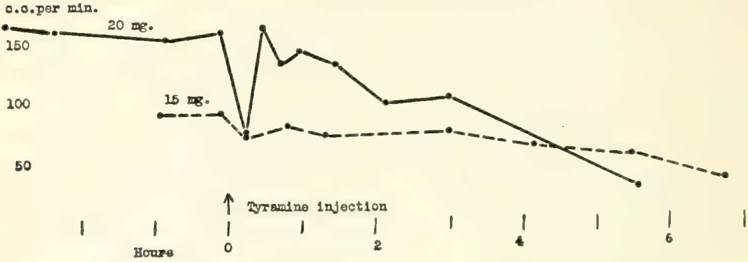
The effects upon the respiratory rate were approximately parallel to the volume changes.

The results of the two larger injections of tyramin are shown in Table 3 and the corresponding volume curves are plotted in Figure 4.

It will be seen that doses of this order are mainly depressant only, the 20 mgm. dose having given immediately a decrease of the respiratory volume by about 50 per cent., a return to normal within the first half-hour, followed again by a less rapid decline. During the marked initial depression excitement, dyspnea, and muscular tremors were noted.

Effects of Morphin and Tyramin Together in the Same Solution.—The effects of introducing morphin and tyramin in the same solution in varying combinations of doses are illustrated in Tables 4, 5 and 6.

Both drugs were given in medium doses (morphin 1 to 1.8 mgm. and tyramin 1.6 to 5.8 mgm. per 100 grams rat), in experiments compiled in Table 4. In twenty-two experiments of this sort it will be seen that carbon dioxid alone produced an increase in the respiratory volume to 135 per cent. of normal (practically the same as in Table 1). The lowest volume determinations after morphin and tyramin in twenty-three experiments were found to average 65 per cent. of normal instead of 40 per cent. of normal when morphin alone was given.



Barbour & Maurer - Fig. 4.

Fig. 4.—Effects of large doses of tyramin upon the respiratory volume. Broken line, rat B 27, 15 mgm. per 100 grams; continuous line, rat B 11, 20 mgm. (per 100 grams). Both injections made at zero hour.

The inefficiency of carbon dioxid in the morphinized animal is again seen in this series of rats, the results showing a minimum determination of 71 per cent. as against 65 per cent. under air breathing.

The experiment illustrated by Figure 5 is typical of the series. Comparison with Figure 2 shows that even though the morphin effect is largely antagonized by tyramin, CO_2 still acts as a very weak stimulus. A reason for this will be suggested later on.

TABLE 4.—EFFECTS OF MEDIUM DOSES OF MORPHIN + MEDIUM DOSES OF TYRAMIN (INCLUDING CO_2 TESTS)

					Respiration, C.c. per Minute						
	Rat					7 to 8 per Cent. CO_2		Morphin + Tyramin		Morphin + Tyramin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Morphin Sulphate, Mg. per 100 Gm.	Tyramin—HCl, Mg. per 100 G'm.	Normal Average	Average	Per Cent. of Normal	Minimum	Per Cent. of Normal	Minimum	Per Cent. of Normal
8/ 6	13	190	1.0	2.1	138	130	94	100	72	117	85
8/10	A6	260	1.0	2.3*	188	240	128	147	78	160	85
8/ 6	12	210	1.0	2.4	132	170	128	87	66	93	70
8/ 9	15	205	1.0	2.4	178	243	125	87	49	93	52
9/21	35	160	1.0	5.0	153	...	...	117	77	...	..
8/ 5	11	140	1.1	5.4	123	163	133	87	71	110	89
8/ 7	14	175	1.1	2.8	140	207	148	73	52	77	55
8/13	21	135	1.1	3.7†	123	200	163	67	54	77	63
8/14	23	130	1.2	3.7	117	200	170	63	54	80	68
8/12	20	125	1.2	4.1	108	183	169	60	56	73	68
8/14	22	120	1.2	4.1	133	183	138	67	50	70	53
8/12	19	135	1.2	4.5	105	147	140	70	67	83	79
8/11	18	130	1.2	4.6	103	137	133	83	81	100	97
8/ 5	10	130	1.2	5.8	103	145	141	43	42	48	47
8/10	16	160	1.3	3.8	78	153	196	60	77	57	73
7/28	8	210	1.4	3.6	177	220	124	97	55	100	56
7/24	3	200	1.5	3.7	183	155	84	100	54	110	60
7/20	1	185	1.6	1.6	140	190	136	97	69	123	88
7/27	6	185	1.6	4.1	140	183	130	128	91	137	98
7/27	7	185	1.6	4.1	143	167	117	140	98	150	105
7/28	9	185	1.6	4.1	167	217	129	93	56	73	43
7/26	5	175	1.7	4.3	193	217	112	107	55	113	59
7/23	2	160	1.8	3.1	153	187	122	100	65	105	69
Average respiratory volume....					140	184	135	90	65	98	71
Average number of respirations per one-quarter minute..					32	33	...	24	..	22	..

* Tyramin repeated after thirty minutes.
† Tyramin repeated after sixty minutes.

The changes in respiratory rate after these morphin-tyramin injections were practically paralled to the volume changes.

In the next series of experiments (Table 5) 1 mgm. per 100 grams rat was taken as the standard dose of morphin, and a more complete antagonism of its action was obtained by giving larger doses of tyramin. For this purpose 6, 10, and 20 mgm. (per 100 grams rat) were chosen. One sees in the table much smaller decreases in the respiratory volume than were noted in Table 4, the average minimum volume being 80 per cent. of normal.

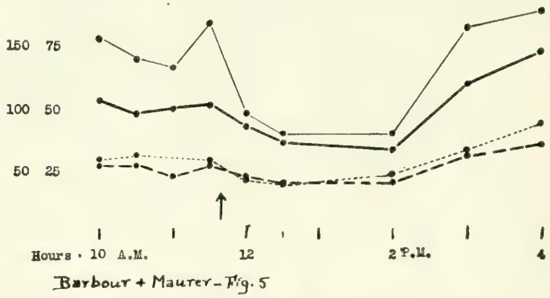


Fig. 5.—Effects of morphin and tyramin injected together in Rat 19, 135 grams. At arrow 1.2 mgm. morphin sulphate and 4.5 mgm. tyramin hydrochlorid (per 100 grams rat) were injected. Heavy lines, air breathing; light lines, CO_2 breathing; continuous lines, respiratory volume in cubic centimeters per minute; broken lines, respiratory rate per one-fourth minute.

TABLE 5.—EFFECTS OF MINIMUM DOSES OF MORPHIN + LARGE DOSES OF TYRAMIN (INCLUDING CO_2 TESTS)

					Respiration, C.c. per Minute						
	Rat					7 to 8 per Cent. CO_2		Morphin + Tyramin		Morphin + Tyramin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Morphin Sulphate, Mg. per 100 Gm.	Tyramin—HCl, Mg. per 100 Gm.	Normal Average	Average	Per Cent. of Normal	Minimum	Per Cent. of Normal	Minimum	Per Cent. of Normal
9/10	24	130	1	6	87	127	146	73	84	97	112
9/10	25	140	1	6	112	190	170	83	73	87	78
9/11	26	185	1	10	168	187	111	123	73	153	91
9/11	27	180	1	10	173	227	131	127	73	143	83
11/12	B20	140	1	10	107	...	...	87	81	...	..
9/15	28	200	1	20	177	213	120	167	94	197	112
9/15	29	230	1	20	193	230	110	167	86	227	118
10/ 8	B1	140	1	20	162	...	...	126	77	...	..
Average respiratory volume....					145	...	131	...	80	...	99
Average number of respirations per one-quarter minute.......					32	33	...	26	..	29	..

It will be noted that the 20 mgm. dose although depressant in itself is a very effective antagonist to morphin depression. We shall revert to this point.

Nothing unusual is seen in the number of respirations per minute in this series or in the degree of stimulation produced by carbon dioxid alone.

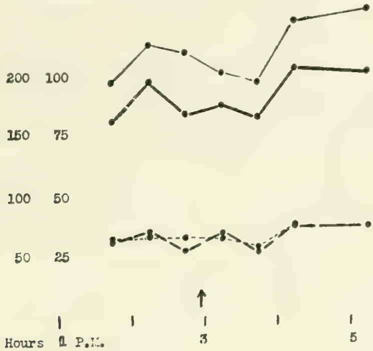


Fig. 6.—Effects of morphin and tyramin injected together in Rat 28, 200 grams. At arrow 1 mgm. morphin sulphate and 20 mgm. tyramin hydrochlorid (per 100 grams rat) were injected. Heavy lines, air breathing; light lines, CO_2 breathing; continuous lines, respiratory volume in cubic centimeters per minute; broken lines, respiratory rate per one-fourth minute.

TABE 6.—EFFECTS OF LARGE DOSES OF MORPHIN + TYRAMIN (INCLUDING CO_2 TESTS)

					Respiration, C.c. per Minute						
	Rat					7 to 8 per Cent. CO_2		Morphin + Tyramin		Morphin + Tyramin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Morphin Sulphate, Mg. per 100 Gm.	Tyramin—HCl, Mg. per 100 Gm.	Normal Average	Average	Per Cent. of Normal	Minimum	Per Cent. of Normal	Minimum	Per Cent. of Normal
7/26	4	150	2	3.3	100	140	140	25	25	30	30
9/18	32	230	2	6.0	169	207	123	150	89	190	112
9/20	33	220	2	6.0	160	200	125	57	36	87	54
Average respiratory volume....					...	...	129	...	50	...	65
Average number of respirations per one-quarter minute... ...					26	27	...	18	..	18	..

One sees, however, after the injections a much greater efficiency of carbon dioxid than was noted in the series just preceding; the average minimum volume under carbon dioxid breathing after the injection of both drugs was in fact 99 per cent. of the normal. This unusual stimulating effect of carbon dioxid in the presence of morphin is well illustrated by an experiment upon Rat 28 (Fig. 6).

Attempts to antagonize larger doses of morphin (2 mgm. per 100 grams) are illustrated in Table 6. The results of three experiments were inconsistent. Comparatively small tyramin doses only were employed.

SUMMARY OF EFFECTS OF MORPHIN, TYRAMIN AND CARBON DIOXID SEPARATELY AND IN VARIOUS COMBINATIONS

The results described up to this point are summarized in Table 7.

The maximum changes in the respiratory volume under the various agents are here tabulated together with the number of experiments (in parentheses) for which they represent the average. It will be seen that 7 to 8 per cent. carbon dioxid increases the respiratory volume of normal rats by about one-third, while morphin in adequate doses diminishes it by three-

TABLE 7.—SUMMARY (1–6)

Table	Morphin, Mg. per 100 Gm. Rat	Tyramin, Mg. per 100 Gm. Rat	Maximum or Minimum Respiratory Volume (Percentage of Normal)*					
			CO_2	Morphin	Tyramin	Morphin + Tyramin	Morphin + CO_2	Morphin + Tyramin + CO_2
1	0.5-2.0	....	136(8)	40(11)	......	.....	43(8)	.....
2	......	1.0–10	.....	......	124(5)	.....	.....	.....
3	......	15.0–20	......	......	56(2)	.....	.....	.....
4	1-1.8	1.6–5.8	135(22)	.....	......	65(23)	.....	71(22)
5	1.0	6.0–20	131(6)	......	......	80(8)	.....	99(6)
6	2.0	3.3–6	129(3)	......	......	50(3)	.....	65(3)

* Number of experiments comprised in each series is indicated in parentheses.

fifths; stimulating doses of tyramin augment the volume (at its maximum) by about one-fourth, the larger doses being depressant. Obviously the best antagonism to morphin is shown in the fifth group of experiments, where 1 mgm. of morphin injected with 6 to 20 mgm. of tyramin reduces the volume by only one-fifth and where the antagonism was often complete at the lowest point under carbon dioxid breathing.

That the efficiency of 7–8 per cent. carbon dioxid as a respiratory stimulant is practically annulled in the morphinized rat, is well illustrated in the first experimental series. Its restoration by small doses of tyramin is seen to be very slight (series IV, 65 → 71 per cent.), but by large doses very pronounced (series V, 80 → 99 per cent.).

CAN THE ANTAGONISM BE ATTRIBUTED TO DELAYED ABSORPTION?

The possibility of tyramin acting to delay the absorption of a drug in the same solution (as is notably done by its congener, epinephrin) must be considered before further discussion of the antagonism. Such an explanation could scarcely apply to the fourth experimental series for such a change in the response to CO_2 as is shown in Figure 5 is proof enough of a rapid absorption of morphin. In connection with the fifth series, however (see Fig. 6), the possible rôle of delayed absorption should be looked into. The question was attacked in a number of different ways: (1) Injections of tyramin were made in mice to determine whether the absorption of dyes was hindered, and secondly whether the absorption of morphin itself was in any way delayed. (2) The respiratory antagonism was studied in rats when the two substances were given on opposite sides of the same animal or at different times. (3) The question as to whether there was a simultaneous antagonism of analgesia was investigated.

1. The Effect of Tyramin on Absorption in Mice.—This was studied by one of us with the assistance of

Dr. W. J. Norris.[7] In two series of experiments in each of which about fifteen mice were used, trypan-blue[8] was injected. The time of appearance of the dye at various parts of the living animal, as well as the depth of color seen at the base of the ears at given time intervals after injection, exhibited the same variations irrespective of whether or not tyramin was included in the injected solution. Similarly, when the drug was given with suitable doses of morphin, no results were obtained demonstrating an effect of tyramin upon the time of appearance or the duration of the Straub tail phenomenon.[9]

2. The Respiratory Antagonism when Morphin and Tyramin are Injected in Different Places Simultaneously or at Different Times.—The results of giving morphin and tyramin simultaneously on opposite sides of the rat are illustrated in Table 8; the results are not summarized, but the individual experiments may be compared with the summary in Table 7. In the case of the second, third, and fourth experiments it appears from Table 8 that the antagonism was not as great as exhibited after similar doses in the Table 5 experiments. The figures apply, however, to a single stage in each case, namely that of the minimum respiratory volume. Reference to Figure 7 (typical of the three experiments mentioned), will show that the morphin depression was brief and was succeeded by a long period of abnormally high respiratory volume, lasting from about the second to the fourth hour. The nature of this curve suggests the possibility of some hindrance to absorption of morphin after the injection of two drugs in the same solution; but such hindrance must have been limited to the first hour.

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[7] Barbour and Norris: Uupublished experiments.

[8] Beyer's "benzo-blue 2B," supplied by courtesy of Drs. P. G. Shipley and Davenport Hooker, was used.

[9] This stimulating effect of morphin (Herrmanns: Biochem. Ztsch. **39**: 221, 1912) was selected as a criterion for absorption rate because it was felt that the chance of its being masked by a true tyramin antagonism was very remote.

The unusual height of the respiratory curve during the later hours of the "opposite sides" experiments suggests the second phase of tyramin action, noted when this drug was given alone.

The two drugs were given at different times in a few experiments which have been compiled in Table 9. The antagonism here was well shown when the tyramin was given some time between twenty minutes before and thirty minutes after the morphin injection, thus again excluding the factor of delayed morphin absorption.

3. Does Tyramin Antagonize the Morphin Analgesia Coincidently with the Antagonism of Respiratory Depression?—The appearance of the customary degree of morphin analgesia after combined injections would, of course, indicate complete absorption of the morphin. On the other hand, coincident antagonism of analgesia and of respiratory depression would prove nothing as to whether or not a delay in absorption had occurred. It would, moreover, not be a surprising event in view of the fact that nearly the same factors would be likely to be involved in these two exhibitions of morphin

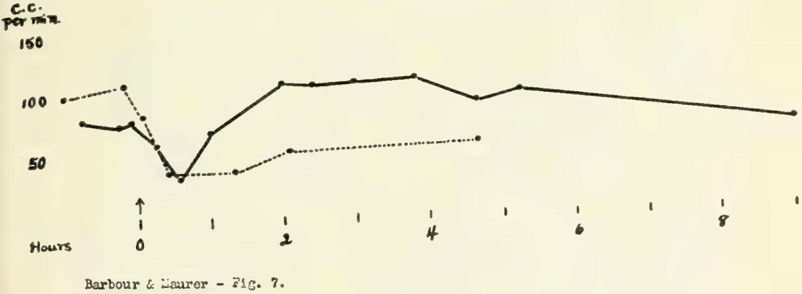

Fig. 7.—Comparison of effects on respiratory volume of morphin alone with those of a simultaneous injection of morphin on one side and tyramin on the other side of a rat. Broken line, rat B 3, morphin 0.5 mgm. (per 100 grams rat); continuous line, rat B 9, morphin 0.5 mgm. per 100 grams on right side of rat and tyramin 20 mgm. per 100 grams on left side of rat. Injections made at zero hour.

action. A retention of morphin analgesia in the presence of a distinct failure of the respiratory depression to appear is of course the combination of events most to be desired from a practical point of view.

The nearest approach to a test of analgesia in animals other than man is of course the observation of objective responses to pain stimuli. These responses were therefore examined in a number of experiments before and after injections of morphin both with and without tyramin. The stimuli selected were pinching the tail at a given point or applying heat to it with a match. The responses observed in the absence of apparent analgesia were both motor and vocal. Below (Protocol II) are illustrated a pair of morphin experiments, one with and the other without tyramin. In the former the usual respiratory depression was noted (minimum volume 36 per cent. of normal). In the latter, where there was a contralateral tyramin injection, is seen a decided respiratory antagonism. The morphin effect here was shallow (minimum volume

TABLE 8.—EFFECTS OF MORPHIN + TYRAMIN GIVEN SIMULTANEOUSLY ON OPPOSITE SIDES OF RAT (INCLUDING CO_2 TESTS)

	Rat				Respiration, C.c. per Minute						
						7 to 8 per Cent. CO_2		Morphin + Tyramin		Morphin + Tyramin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Morphin Sulphate, Mg. per 100 Gm.	Tyramin—HCl, Mg. per 100 Gm.	Normal Average	Average	Per Cent. of Normal	Minimum	Per Cent. of Normal	Minimum	Per Cent. of Normal
10/26	B9	155	0.5	20	83	...	...	32	39	...	..
9/18	31	230	1.0	6	160	170	106	80	50	117	73
11/17	B23	115	1.0	10	90	...	...	64	71	...	..
10/11	B2	130	1.0	20	111	...	...	66	59	...	..
9/20	34	230	2.0	6	133	170	128	30	22	37	27

59 per cent. of normal) and very transitory and succeeded by a prolonged respiratory stimulation (maximum 130 per cent. of normal). The protocol should be compared with Figure 7.

It will be noted from the control experiment that our smallest dose of morphin alone, once its full action was exhibited, sufficed to give a complete analgesia, lasting for several hours. In the tyramin-morphin experiment also, during the period of temporary respiratory depression, the response to pinching was completely eliminated, but that to heat persisted to a slight extent. Of chief significance was the fact that during the subsequent period of abnormally high respiratory volume a very clear indication of morphin analgesia was retained. Similar effects have been noted in other rats. We have demonstrated therefore that the objective manifestations of a partial morphin analgesia can be retained during complete and prolonged antagonism of the respiratory effects of this drug.

TABLE 9.—EFFECTS OF MORPHIN + TYRAMIN GIVEN AT DIFFERENT TIMES (INCLUDING CO_2 TESTS)

	Rat					Respiration, C.c. per Minute						
							7 to 8 per Cent. CO_2		Morphin + Tyramin		Morphin + Tyramin + 7 to 8 per Cent. CO_2	
Date	Number	Weight, Gm.	Morphin Sulphate, Mg. per 100 Gm.	Tyramin—HCl, Mg. per 100 Gm.	Tyramin Given Before or After Morphin Minutes	Normal Average	Average	Per Cent. of Normal	Minimum	Per Cent. of Normal	Minimum	Per Cent. of Normal
9/23	37	175	1	25	60b	157	207	132	63	40	77	48
11/15	B21	110	1	10	30b	81	...	...	21	26	...	..
9/23	36	170	1	5	20b	170	210	123	147	66	127	74
9/16	30	200	1	5	15a	145	183	125	90	61	117	79
10/12	B 5	190	1	20	30a	101	...	...	73	72	...	..
11/15	B22	125	1	20	120a	72	...	...	25	35	...	..

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Rat B3 (120 Grams)

Time	Expired per Minute C.c.	Rate per Minute	Remarks
2:46–2:49 p. m.	114	160	Never had a drug before
2:50–2:53 p. m.	102	168	
3:38–3:41 p. m.	114	156	
3:43–3:46 p. m.	116	168	Lively responses
3:49 p. m.	...	...	Morphin sulphate (subcutaneously) 0.6 mgm. (=0.5 mgm. per 100 gm. rat)
3:51–3:54 p. m.	89	124	Poor responses to pinching and heat
4:13–4:16 p. m.	41	52	
5:02–5:07 p. m.	40	68	No responses
5:09–5:12 p. m.	42	64	
5:52–5:55 p. m.	60	72	
5:56–5:59 p. m.	55	...	
8:22–8:25 p. m.	56	80	No responses
8:27–8:30 p. m.	61	...	
Normal average	112	163	
Minimum	40	64	1¼ hours after injection
Per cent. of normal	36	39	
Last observation, per cent. of normal	55	...	4⅔ hours after injection

Rat B2 (130 Grams)

Time	Expired per Minute C.c.	Rate per Minute	Remarks
2:04–2:07 p. m.	109	168	Never had a drug before
2:08–2:11 p. m.	113	172	
2:57–3:00 p. m.	108	168	
3:01;3:04 p. m.	115	160	Lively responses
3:07 p. m.	...	...	p. oxy. HCl (subcutaneously right side) 26 mgm. (=20 mgm. per 100 gm. rat) Morphin sulphate (subcutaneously left side) 1.3 mgm. (=1 mgm. per 100 gm. rat)
3:12–3:15 p. m.	115	172	
3:17–3:20 p. m.	85	96	Irregular respiration
3:22–3:25 p. m.	91	104	Irregular respiration; responses greatly diminished
3:28–3:31 p. m.	87	104	
3:59–4:02 p. m.	66	80	No response to pinching, poor response to heat
4:47–4:50 p. m.	114	112	
4:55–4:58 p. m.	119	...	Responses a little better
5:21–5:24 p. m.	144	120	
5:25–5:28 p. m.	136	136	Responses improved but not nearly normal
6:15–6:18 p. m.	132	128	
6:18–6:20 p. m.	139	...	
8:33–8:36 p. m.	117	152	
8:37–8:40 p. m.	121	...	
Normal average	111	167	
Minimum	66	80	55 minutes after injection
Per cent. of normal	59	48	
Maximum	144	...	
Per cent. of normal	130	...	2¼ hours after injection
Last observation, per cent. of normal	109	...	4½ hours after injection

DISCUSSION OF THE NATURE OF THE RESPIRATORY ANTAGONISM

It will be recalled that the most pronounced phase of the tyramin respiratory stimulation is delayed until the second hour or later. This was seen under various doses of the single drug (Fig. 3) as well as in connection with the contralateral morphin injections (Fig. 6 and 7 and Protocol II). We know that the tyramin-molecule is readily decomposed in the body [10] and can scarcely play the chief rôle in an action the duration of which is measured in hours. The late phase of stimulation suggests therefore either a prolonged action of the metabolism resulting in the production of stimulating substances or else the gradual liberation in the body of decomposition products of tyramin itself. Something of the nature of this stimulating agent or agents may perhaps be learned by a closer examination of our results with carbon dioxid.

The well-known failure of carbon dioxid to stimulate the respiration after morphin appears to be due to the increase in the alkali reserve of the blood induced by the latter.[11] Under the conditions of our experiments, 7.5 per cent. of CO_2 in the inspired air, which served to increase the rsepiratory volume by 35 or 36 per cent. in normal animals, sufficed only to raise it by 3 per cent. of the normal volume in the morphinized animal (see Table 7). This indicates that the alkali reserve was so increased that 7.5 per cent. was but little in excess of the alveolar concentration in such an animal.

Let us next employ the results of CO_2 breathing as an indicator of the alkali reserve of the blood in the tyramin animals. We note from an experiment (Rat A1) in Table 2, that a small dose of tyramin in a normal animal exerted no demonstrable effect upon the alkali reserve. (There was a simple summation of the separate effects of tyramin and carbon dioxid when the two stimuli were acting together.)

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[10] Ewins, A. J., and Laidlaw, P. P.: J. Physiol., 1910, 41-78.

[11] Hjort, A. M., and Taylor, F. A.: J. Pharm. & Exp. Therap. **13:** 407, No. 5, 1919.

In the morphin-tyramin animals there was noted after the lower doses of tyramin a very feeble stimulating effect from CO_2 (Fig. 5). These animals therefore maintained an alkali reserve at about the same level as that induced under similar conditions by morphin alone. That the tyramin however kept the figure for the minimum respiratory volume at an average height of 65 per cent. as against 40 per cent. after morphin alone, indicates the presence of a potent non-acid stimulating factor. This would exclude metabolic hormones and point to the tyramin molecule or non-acid by-products, the capacity to stimulate the respiration perhaps residing chiefly in the benzene nucleus or the amino group.

On the other hand, given with the larger doses of tyramin (Fig. 6) morphin seems to have but little effect in impairing the stimulating capacity of CO_2. From this it must be inferred that tyramin is here tending to keep down the alkali reserve. As the causative agent metabolites resulting from marked cardaic stimulation suggest themselves as well as the p-oxyphenyl-acetic acid liberated from the tyramin molecule.[12] Evidently substantial doses of tyramin are necessary for this effect.

This inference that acid products play an important rôle in the action of large doses of tyramin will account for certain of our results which at first sight appear conflicting. We refer to the difference in the behavior of the respiration after 20 mgm. (per 100 grams) of tyramin according to whether or not the animal had been morphinized. This dose, although depressing in itself (Table 3), antagonizes morphin depression (Table 5). Excess of acid products presumably converts the small dose excitant effect of tyramin into the large dose depressant effect. The better alkali reserve obtaining under morphin protects against this excess and thus a favorable H-ion concentration for a high respiratory volume is afforded by the large tyramin dose.

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[12] Cf. also the observation of Barbour, Maurer and von Glahn (loc. cit.) that tyramin augments oxidations in man.

The assumption of at least two factors therefore seems necessary in explaining the stimulating action of tryramin. First, to account for stimulation in the presence of a high alkali reserve may be suggested an action of its own molecule or some decomposition product of a non-acid nature. Secondly, to account for the modifications induced by morphin and by CO_2, an acid factor is also postulated in the case of large doses.

Further studies with special reference to the neutrality regulation should afford a basis for practical applications of the tryamin-morphin antagonism.

CONCLUSIONS

1. The open-circuit apparatus described is suitable for complete respiratory studies in unanesthetized rats. The following conclusions apply to these animals.

2. Seven to eight per cent. carbon dioxid breathing increases the respiratory volume by about one-third, the rate remaining practically unchanged.

3. Morphin sulphate (0.5 to 2 mgm. per 100 grams) diminishes the respiratory volume by about three-fifths of the normal; the rate shows a smaller decrease. Four, five, or more hours are required for a return to normal conditions.

4. Tyramin hydrochlorid (1 to 10 mgm. per 100 grams) produces in the curve of respiratory volume a prolonged though moderate increase. During the earliest hours of the stimulation there often occurs a temporary relapse to the normal level or even below. The average maximum volume represents a 25 per cent. increase over the normal. Doses larger than 10 mgms. are mainly depressant in their action.

5. Tyramin (1.6 to 20 mgm. per 100 grams) antagonizes the respiratory effects of morphin (1 to 1.8 mgm. per 100 grams) when the two are injected

in the same solution. The most complete antagonism was noted when 1 mgm. of morphin was injected with 6 to 20 mgm. tyramin, the average maximum decrease from normal volume amounting to only one-fifth.

6. Carbon dioxid (7 to 8 per cent.) breathing has practically no stimulating effect upon morphinized rats. It increases the stimulating effect of tyramin under normal conditions. After morphin carbon dioxid (7 to 8 per cent.) potentiates the tyramin antagonism where optimum doses of the latter are employed, but has a very feeble effect when the tyramin doses are smaller.

7. Tyramin-morphin antagonism is not due to a delay caused by the former drug in the absorption of the latter.

8. Simultaneous injection of morphin and tyramin in separate subcutaneous regions results in a greater exhibition of respiratory depression than when the drugs are given in the same solution but this is followed promptly by an unusually marked antagonism.

9. The objective manifestations of a partial morphin analgesia can be retained during complete and prolonged antagonism of the respiratory depression.

10. To account for all of the results obtained it appears necessary to assume both a non-acid and an acid factor in the stimulating action of tyramin.

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PREVENTION OF SIMPLE GOITER IN MAN

FOURTH PAPER *

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(*From Archives of Internal Medicine, June* 15, 1920)

In previous publications[1] we have outlined the plan of prevention, presented the data of the incidence of thyroid enlargements as determined by annual surveys of all new pupils in the Akron public schools, and the results of the prophylactic use of sodium iodid for nineteen months. The present paper deals with the data obtained at the fourth general examination made October 13-17, 1919, together with summaries and conclusions based on observations extending over a period of thirty months.

ANALYSIS OF THE RECORDS OF NEW PUPILS

The general data of the clinical condition of the thyroid gland are given in Table 1. For comparison and reference the figures for the three previous examinations are also given.

The pupils included in this table are new admissions to all grades from the fifth to the twelfth, inclusive, and presumably had not previously received iodin. The figures in the first line represent the results of the original survey of all girls in grades from the fifth to the twelfth, inclusive. The figures in the second line include (1) incoming fifth grade girls, (2) girls entering grades above the fifth grade, and (3) girls of two schools that accidentally lost the records of those not taking the treatment. The figures in the third and fourth lines include (1) incoming fifth grade girls and (2) girls entering grades above the fifth. The progressive increase in the percentage of normal thyroids (43.6, 47.0, 55.4 and 65.4) and the corresponding progressive decrease in the percentage of enlarged thyroids whether taken together (56.4, 53.0, 44.6 and 34.5) or as separate groups (slightly enlarged, moderately enlarged and markedly enlarged) are due to the increasing preponderance of fifth grade girls in the second, third and fourth groups. This is also shown in Table 2, where all new pupils are grouped according to ages. Fifth grade pupils average from 10 to 11 years of age, and approximately 95 per cent. are included in the age group from 10 to 12. Details of the relation of age to the clinical condition of the thyroid are given in Tables 3, 4 and 5.

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* From the Department of Experimental Medicine, Western Reserve University.

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[1] Marine, D., and Kimball, O. P.: The Prevention of Simple Goiter in Man, J. Lab. & Clin. Med. **3**: 40, 1917. Kimball, O. P., and Marine, D.: The Prevention of Simple Goiter in Man (Second Paper), Arch. Int. Med. **22**: 41 (July) 1918. Kimball, O. P., Rogoff, J. M., and Marine, D.: The Prevention of Simple Goiter in Man (Third Paper), J. A. M. A. **73**: 1873 (Dec. 20) 1919.

TABLE 1.—ANALYSIS OF THE RECORDS OF NEW PUPILS

Date Examined	Total Cases Examined	Total New Cases	Normal		Slight Enlargements		Moderate Enlargements		Marked Enlargements		Adenomas	
			No.	%	No.	%	No.	%	No.	%	No.	%
April 1917....	3,872	3,872	1,688	43.6	1,931	49.9	246	6.3	7	0.2	39	1.0
November 1917........	4,415	1,772	831	47.0	820	46.2	121	6.8				
November 1918........	4,277	1,873	1,037	55.4	779	41.6	53	2.8	4	0.2	6	0.3
October 1919........	4,520	2,162	1,415	65.4	679	31.4	67	3.1	1	0.05	1	0.05

For reference and comparison the original survey of all pupils (April, 1917) is given in Table 3, while in Tables 4 and 5 are given the results of the surveys of new pupils for 1918 and 1919, respectively. It should be emphasized, that in the 1917 examination, 43.9 per cent. of the girls in the 10-12 years age group had enlarged thyroids; that in the 1918 examination 35.8 per cent. had enlarged thyroids; and that in the 1919 examination 23.2 per cent. had thyroid enlargements.

TABLE 2.—SUMMARY OF AGE INCIDENCE—NEW PUPILS

		Age									
		10–12		12–14		14–16		16–18		18–20	
Date of Examination	Total New Cases	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
April 1917..	3,872	945	24.4	1,261	32.6	1,140	29.4	453	11.7	73	1.0
November 1918......	1,873	766	40.9	590	31.5	406	21.7	94	5.0	17	0.9
October 1919......	2,162	969	44.8	678	31.4	401	18.6	102	4.7	12	0.5

TABLE 3.—RELATION OF AGE TO THYROID CONDITION—NEW PUPILS—1917

	Age									
	10–12		12–14		14–16		16–18		18–20	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal...........	530	56.1	521	41.3	460	40.3	156	34.4	21	28.8
Slightly enlarged	394	41.7	680	53.9	578	50.7	235	51.9	44	60.3
Moderately enlarged..........	21	2.2	59	4.7	98	8.6	60	13.2	8	11.0
Markedly enlarged..........	...	....	1	0.1	4	0.3	2	0.4		

TABLE 4.—RELATION OF AGE TO THYROID CONDITION—NEW PUPILS—1918

	Age									
	10–12		12–14		14–16		16–18		18–20	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal...........	491	64.2	295	50.0	214	52.7	39	41.5	9	52.9
Slightly enlarged	267	34.8	276	46.8	168	41.4	49	52.1	6	3.53
Moderately enlarged..........	8	1.0	19	3.2	24	5.9	6	6.4	2	11.8
Markedly enlarged..........	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

TABLE 5.—RELATION OF AGE TO THYROID CONDITION—NEW PUPILS—1919

	Age									
	10–12		12–14		14–16		16–18		18–20	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal...........	743	76.7	419	61.8	199	49.6	53	51.9	7	58.3
Slightly enlarged	215	22.2	239	35.3	170	42.4	43	42.2	5	41.7
Moderately enlarged..........	11	1.1	20	2.9	31	7.8	6	5.9	0	0.0
Markedly enlarged..........	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0

This is important from the standpoint of the age at which the prophylactic treatment should be started. When this work was begun, no data of this kind were available, and the fifth grade was arbitrarily chosen as the lower limit, because our limited facilities made it necessary to confine our efforts to what seemed to be the most important age periods. We have seen only forty instances of moderately enlarged glands and no instance of marked enlargement in the 10-12 years age group, and as very striking therapeutic effects are seen in these slight hyperplasias it makes little difference in the ultimate result. If, however, one had to depend entirely on prevention it would be necessary to begin at an earlier age.

There appears to be no noteworthy difference in the incidence of thyroid enlargements between white and colored children. The data are, however, insufficient for any definite conclusion. The data on the twenty-seven colored children are given in Table 6.

TABLE 6.—ANALYSIS OF RECORDS OF NEW PUPILS—NEGROES—1919

	Age					
	10–12		12–14		14–16	
	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.
Normal	5	50.0	7	53.9		
Slightly enlarged	4	40.0	5	38.5	3	75.0
Moderately enlarged	1	10.0	1	7.6	1	25.0

EFFECT OF PROPHYLACTIC TREATMENT

The prophylactic treatment as carried out for the past two years consists of the administration of 2 gm. sodium iodid, given in 0.2 gm. doses daily, for ten consecutive school days, repeated each spring and autumn. The general data of those pupils not taking the treatment are given in Table 7, and of those taking the treatment in Table 8. Only pupils with two or more consecutive examinations have been included in the tabulations. A considerable number of pupils, both taking and not taking the treatment, have been omitted

TABLE 7.—RECORD OF PUPILS NOT TAKING PROPHYLACTIC TREATMENT

Time Under Observation, Mos.	Normal				Slightly Enlarged						Moderately Enlarged					
	Unaltered		Increased		Unaltered		Increased		Decreased		Unaltered		Increased		Decreased	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
6	47	50.0	47	50.0	93	69.4	36	26.9	5	3.7	16	69.6	7	30.4	0	0.0
12	420	75.5	136	24.5	251	70.3	35	9.8	71	19.9	17	65.4	8	30.8	1	3.8
18	103	65.2	55	34.8	108	74.5	18	12.3	19	13.1	11	57.9	3	15.8	5	26.3
24	135	76.7	41	23.3	106	79.7	8	6.0	19	14.3	9	60.0	3	20.0	3	20.0
30	205	75.1	68	24.9	140	73.7	30	15.8	20	10.5	4	66.7	0	0.0	2	33.3

TABLE 8.—RECORD OF PUPILS TAKING PROPHYLACTIC TREATMENT

Time Under Observation, Mos.	Normal				Slightly Enlarged						Moderately Enlarged					
	Unaltered		Increased		Unaltered		Increased		Decreased		Unaltered		Increased		Decreased	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
6	17	94.4	1	5.6	54	69.2	1	1.3	23	29.5	9	81.8	0	0.0	2	18.2
12	344	99.7	1	0.3	187	45.5	0	0.0	224	54.5	10	23.8	0	0.0	32	76.2
18	73	100.0	0	0.0	72	52.3	1	0.7	64	46.7	7	28.0	0	0.0	18	72.0
24	184	100.0	0	0.0	72	37.9	1	0.5	117	61.6	2	7.7	0	0.0	24	92.3
30	288	100.0	0	0.0	92	28.5	0	0.0	231	71.5	1	2.6	0	0.0	38	97.4

because they missed one examination, although otherwise their records were complete. Two thousand, three hundred and five pupils are included in the tabulation of those not taking treatment, and 2,190 in the tabulation of those taking treatment. Further, it was necessary to tabulate the results with reference to the length of time under observation. As the prophylactic treatment was given at intervals of six months, we have used this interval as the unit and grouped the pupils according to the periods under observation, 6, 12, 18, 24 and 30 months respectively. Only the results of three groups, (normals, slightly enlarged, and moderately enlarged) are included because the fourth group (markedly enlarged) is too small. A comparison of the two tables brings out striking differences between those not taking and those taking iodin. These differences are manifested both in *prevention* of enlargement and in a *decrease* in the size of existing enlargements, i.e., therapeutic effect.

TABLE 9.—SUMMARY—RECORDS OF PUPILS TAKING AND NOT TAKING PROPHYLACTIC TREATMENT

	Taking		Not Taking	
	Totals	Per Cent.	Totals	Per Cent.
Normal:				
Unchanged	906	99.8	910	72.4
Increased	2	0.2	347	27.6
Slightly Enlarged:				
Unchanged	477	41.9	698	72.8
Increased	3	0.3	127	13.3
Decreased	659	57.8	134	13.9
Moderately Enlarged:				
Unchanged	29	20.3	57	64.0
Increased	0	0.0	21	23.6
Decreased	114	79.7	11	12.4
Total	2,190	....	2,305	

Prevention.—This effect is shown in the columns marked "unchanged" and "increased." Taking the totals for the five six-month periods (Table 9), the following results were obtained. Of those that were normal at the first examination and did not take iodin, 347, or 27.6 per cent., have enlarged thyroids, while of those that were normal at the first examination and took iodin as outlined, two, or 0.2 per cent., have enlarged thyroids. These two instances of enlargement were investigated. The first pupil, M. T., age 16, had her thyroid examined and classified as normal May 2, 1917, Oct. 17, 1918 and Dec. 3, 1918. At the examination Oct. 15, 1919, it was classified as slightly enlarged. This girl had taken 2 gm. of sodium iodid during each of the five possible periods, May, 1917, November, 1917, May, 1918, December, 1918 and May, 1919. A special examination was made Jan. 13, 1920. The enlargement of the thyroid was verified. The enlargement was acquired as opposed to congenital, as shown by the absence of a pyramidal process or thyroglossal tract. The tonsils were markedly enlarged, nearly meeting in the midline when the mouth was widely opened. They were abnormally hyperemic, and on direct questioning the pupil stated she was subject to recurrent tonsillitis. There was also slight enlargement of the lymphoid tissue at the base of the tongue and in the nasopharynx. The general impression was that of a neurotic individual with general lymphoid hyperplasia.

The second girl, aged 15, had her thyroid first examined and classified as normal Nov. 27, 1918. At the examination Oct. 16, 1919, it was classified as slightly enlarged. This girl had taken 2 gm. sodium iodid during each of the two available periods, November, 1918, and May, 1919. A special examination was made Jan. 13, 1920, and the thyroid enlargement was verified. Careful inspection revealed the presence of Hutchinson teeth, depressed nasal arch and interstitial keratitis. We considered the case one of neglected congenital syphilis.

Passing to Group 2, or those classified as having slightly enlarged thyroids at the first examination, it is seen among those not taking the prescribed treatment that 127, or 13.3 per cent., underwent further enlargement, while of those taking the prescribed treatment, three, or 0.3 per cent., underwent further enlargement. Two of these three pupils were again examined Jan .13, 1920. One, R. R., aged 14, was examined May 2, 1917, Oct. 12, 1917, and Nov. 26, 1918, and the thyroid was classified as slightly enlarged, and at the examination Oct. 16, 1919, the gland was classified as moderately enlarged. This girl had taken the prescribed treatment only during the last three available periods, May, 1918, November, 1918, and May, 1919. A special examination was made Jan. 13, 1920, and the thyroid enlargement verified. In this case also the tonsils were enlarged and the seat of recurrent infections. The second case, V. S., aged 11, was examined Oct. 22, 1917, and Nov. 27, 1918, and the thyroid classified as slightly enlarged. At the third examination, Oct. 16, 1919, it was classified as moderately enlarged and the special examination Jan. 13, 1920, verified this finding. This girl had taken the prescribed treatment during the four available periods, November, 1917, May, 1918, December, 1918, and May, 1919. Superficial inspection failed to reveal the existence of any associated pathologic condition as was found in each of the first three cases mentioned. The fifth girl was not present for the special examination. These five cases are the only instances out of 2,190 pupils taking iodin that showed enlargement. For the group with slightly enlarged thyroids taking iodin, 447, or 41.9 per cent., remained unchanged, while of those not taking iodin, 698, or 72.8 per cent., remained unchanged.

Passing to the third group, or those classified as having moderately enlarged thyroids at the first examination, it is seen that of those taking iodin, twenty-nine, or 20.3 per cent., remained unchanged, while of those not taking iodin, fifty-seven, or 64.0 per cent., remained unchanged; of those taking iodin none increased, while of those not taking it, twenty-one,or 23.6 per cent., increased.

Curative or Therapeutic Effect.—Although of secondary importance, the results are just as striking as those above described under prevention. These results are shown in the column marked "decreased." Of those pupils whose thyroids were classified as slightly enlarged at the first examination, and who took iodin, 659, or 57.8 per cent., definitely decreased in size, while of those not taking the prescribed treatment, 134, or

13.9 per cent., decreased. Passing to the group whose thyroids were classified as moderately enlarged at the first examination, 114, or 79.7 per cent., of those taking iodin showed definite decreases. In some the decrease in size was most striking and hardly to be believed had we not had actual measurements and descriptions of the condition previously. The reduction in several cases was as marked as one sees in the thyroid enlargement of young dogs, sheep or cattle following the use of iodin. It means that with similar anatomic conditions, i.e., uncomplicated hyperplasias of the thyroids, the degree of reaction is similar. Ordinarily, one does not obtain the striking therapeutic effect on human thyroid enlargements that is seen in animals This, as pointed out in previous papers, is due, in large part, to the duration of the enlargement, the presence of adenomas, cysts, degenerations, hemorrhage, etc., which are common in all long standing human goiters, while very uncommon in the lower animals at the ages when these animals are usually observed. The therapeutic effect is a very important supplement to prevention and makes it possible to begin prophylactic treatment in older pupils with the same practical result than would otherwise be possible.

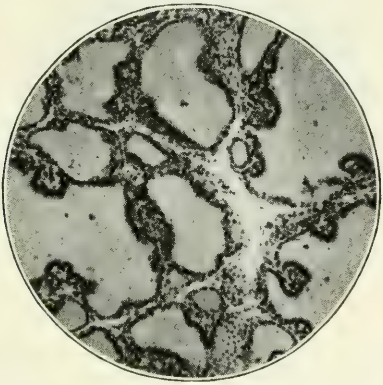

Fig. 1.—Moderate active hyperplasia; control specimen removed ten days after beginning administration of iodin (D— 185a).

DISCUSSION

Our observations on the prevention of simple goiter in man have extended over a period of thirty months. The disease is as easily prevented in man as in fish or in domestic animals.

Of 2,190 pupils taking 2 gm. sodium iodid twice yearly, five have shown enlargement of the thyroid, while of 2,305 pupils not taking the prophylactic, 495 have shown enlargement of the thyroid. Of 1,182 pupils with thyroid enlargement at the first examination and who took the prophylactic, 773 thyroids have decreased in size, while of 1,048 pupils with thyroid enlargement at the first examination and who did not take the prophylactic, 145 thyroids have decreased in size. These figures demonstrate in a striking manner both the preventive and the therapeutic effects. There is an error in the above figures in that many pupils listed as not taking iodin have taken iodin in one or another form outside the school jurisdiction. No attempt has been made to detect or estimate this error.

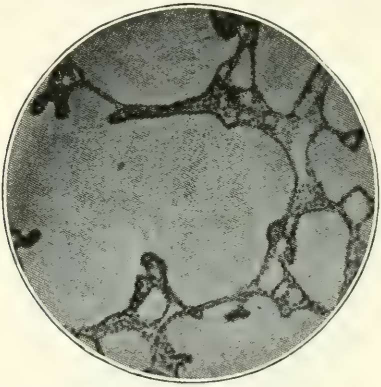

Fig. 2.—Same gland as Figure 1; specimen removed forty-four days after beginning administration of iodin.

In the practical application of the preventive treatment, one must keep in mind the three periods when simple thyroid enlargements most commonly occur, viz., (1) fetal, (2) adolescence and (3) pregnancy.

(1) Prevention of goiter in mother and fetus is as simple as that occurring during adolescence. Practically, it would seem that it is a charge or responsibility of individual members of the medical profession supplemented with public education.

(2) The prevention of goiter of adolescence, on the other hand, should be a public health measure under state, county or municipal control. The existing systems of organization of the schools, public and private, is sufficient to handle all the details without additional aid or expense. Education of the pupils would be combined with the actual administration so that after leaving school they could continue the treatment, if necessary. Physicians in industrial medicine could render an important service in this field. Thyroid enlargement is approximately six times as frequent in girls as in boys. It is a social economic question each community must decide whether it will include both sexes. Likewise, as to the age of beginning and stopping the use of iodin. In this climate probably the maximum of prevention, coupled with the minimum of effort, would be obtained by giving it between the ages of 11 and 17 years. As applied to our schools it would mean beginning with the fifth grade.

Manner and Form of Administration.—As previously stated, iodin is taken up by the thyroid gland when given by mouth, inhalation, or by external application. Weith[2] reports favorable therapeutic effects from inhalation of iodin as carried out by suspending a wide mouthed bottle containing a 10 per cent. tincture of iodin in the school room. Waste and lack of control of amounts taken are the most obvious objections. Similar objections hold in case of external application. Some form of oral administration seems most practical and economical. The addition of iodin or a salt of iodin to the water supply as we have done in preventing goiter in fish might be considered. There are obvious objections to such a plan. It would entail enormous waste. It is applicable only when there are installations, i.e., in towns and cities, and depending on the chemical impurities in water interactions might throw out the iodin. The most feasible oral method would seem to be the individual administration of definite small amounts, either in solution or as tablets. The cheapest salt, sodium iodid, could be given in either form. Manufacturing pharmacists state that sodium iodid could be prepared very cheaply in tablet form protected from the action of water and light. For private use, the well known U.S.P. preparations, syrup of ferrous iodid and syrup of hydriodic acid are excellent.

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[2] Weith: Goiter and Iodin in the School, Cor.-Bl. f. schweiz. Aerzte **49**: 1474, 1919.

Amounts of Iodin to be Used.—An ounce of syrup of ferrous iodid or hydriodic acid given over a period of from two to three weeks and repeated twice yearly would seem ample. As a public health measure, we have used 2 gm. of sodium iodid given over a period of two weeks and repeated twice yearly. This dosage has prevented enlargement of the thyroid in more than 99 per cent. of the children in this mildly goiterous district. It is our opinion that much smaller amounts would suffice for healthy children and healthy pregnant women, provided the period of taking was prolonged, i. e., 1 gm. sodium iodid distributed over a month would accomplish as good thyroid effects as 2 gm. given over a period of two weeks.

The prevention of thyroid enlargement in individuals with other diseases or residing in extremely goiterous districts, as in some glacial valleys of Alaska and British Columbia; certain districts in the Alps and Himalayas, might require larger amounts of iodin for normals than above indicated. Our data of the clinical condition of four of the five cases that enlarged during the administration of 2 gm. of sodium iodid, twice yearly, suggest that infectious (chronic catarrhal or suppurative tuberculosis, syphilis, etc.) and possibly also in conditions like chlorosis, osteomalacia, lymphatism and exophthalmic goiter, such amounts might not control the thyroid growth. In such conditions there may be a greatly increased demand for the thyroid hormone or the organism's ability to store iodin in the thyroid may be impaired. There is a great deal of clinical evidence for the first view and none at present in support of the second.

Effect of Iodin on the Thyroid Gland.—This is manifested in two ways (1) on the iodin store and (2) on the histologic condition. Both of these effects have been fully described in previous papers.[3]

Effect on the Store: If the thyroid gland is not saturated with iodin (i. e., contains less than 4 mg. per gm. of dried gland) it is taken up readily by the cells following its administration in any form and in any manner thus far studied. An increase in the iodin content of thyroid may be demonstrated in a few seconds following the injection of a soluble salt into the circulation.[4] Iodin thus taken up is held by the cells until elaborated into the physiologically active hormone, when any excess is excreted into the follicular spaces and stored in the so-called colloid. Two factors then are concerned in the storage of iodin in the thyroid: (a) the capacity of the gland cells to take up and elaborate the hormone and (b) the capacity of the colloid material to store the product. It is evident, then, that to obtain maximum thyroid effects from a minimum amount of iodin, it should be administered in amounts not to exceed the capacity of the cells at any given time to handle it. As has been shown, the elaboration of the hormone proceeds slowly[5] in the most active thyroids. Also when one recalls that from 4 to 5 mg.

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[3] Marine, D.: On the Occurrence and Physiological Nature of Glandular Hyperplasia of the Thyroid (Dog and Sheep) Together with Remarks on Important Clinical Human Problems, Bull. Johns Hopkins Hosp. **18**: 359, 1907. Marine, D., and Lenhart, C. H.: Colloid Glands (Goiters): Their Etiology and Physiological Significance, Bull. Johns Hopkins Hosp. **20**: 131, 1909. Marine, D., and Lenhart, C. H.: Effects of Administration or the Withholding of Iodin Containing Compounds in Normal, Colloid or Actively Hyperplastic Thyroids of Dogs, Arch. Int. Med. **4**: 253, 1909. Marine, D.: Quantitative Studies on the in vivo Absorption of Iodin by Dogs' Thyroid Glands, J. Biol. Chem. **22**: 547, 1915.

[4] Marie, D., and Rogoff, J. M.: The Absorption of Potassium Iodid by the Thyroid Gland in vivo Following Its Intravenous Injections in Constant Amounts, J. Pharm. & Exper. Therap. **8**: 439, 1916.

[5] Marine, D., and Rogoff, J. M.: How Rapidly Does the Intact Thyroid Gland Elaborate Its Specific Iodin Containing Hormone? J. Pharm. & Exper. Therap. **9**: 1, 1916.

of iodin per gm. of dried gland, or from 25 to 30 mg., is the total storage capacity of a normal thyroid, it is clear that small amounts of iodin (a few mg.) given daily for a long period of time (a month or more) would produce optimum thyroid effects. In the school work, a compromise was found necessary, increased amounts and decreased time of administration.

Effect on Histology of the Thyroid: It has been shown that the minimum amount of iodin store necessary to maintain normal or quiescent thyroid structure is quite constant for mammals.[6] In the dog, sheep, human and pig thyroid it is approximately 1 mg. per gm. of dried gland, and immediately the percentage is reduced below the minimum, hypertrophic and hyperplastic changes begin and continue until the store of iodin has again been raised above the minimum requirements, when involution takes place. This cycle may be repeated many times in the same individual under natural or experimentally controlled conditions. In young

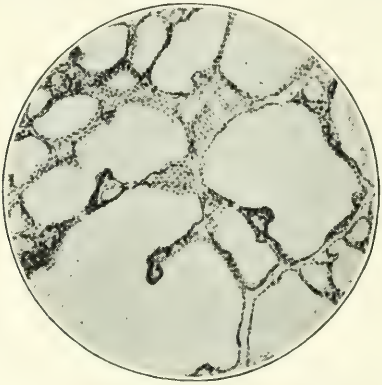

Fig. 3.—Same gland as Figures 1 and 2; specimen removed seventy-eight days after beginning administration of iodin. Total amount of iodin given, 90 mg.

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[6] Marine, D., and Williams, W. W.: Relation of Iodin to the Structure of the Thyroid Gland, Arch. Int. Med. **1**: 349, 1908. Marine, D., and Lenhart, C. H.: Further Observations on the Relation of Iodin to the Structure of the Thyroid Gland in the Sheep, Dog, Hog and Ox, Arch. Int. Med. **3**: 66, 1909.

dogs, with active hyperplasia, involution is usually complete in from fourteen to twenty-one days after beginning the administration of iodin. The histologic features of this involution have been described in detail in other papers, but for reference three microphotographs illustrating it are reproduced (Figs. 1, 2 and 3).

Untoward Effects.—No obvious case of exophthalmic goiter has developed, although such cases have been carefully looked for. An occasional instance of iodin idiosyncrasy (iodism), amounting to less than 0.5 per cent. of the cases, was noted. Most of the cases were very mild and the girls did not stop the treatment. As an untoward effect it is negligible.

SUMMARY

Observations on the prevention of simple goiter in man on a large scale have extended over a period of thirty months. The results show that it may be prevented very simply and cheaply in normal individuals. While thyroid enlargements of adolescence are more common, they are not more important than those occurring in mother and fetus. Prevention of adolescent goiter is properly a public health problem, while the prevention of fetal and maternal thyroid enlargements is largely a responsibility of individual physicians. The presence of pathologic conditions may modify the result of the prophylactic treatment in individual cases. While such instances are rare, they are important and merit detailed reports.

REPORT OF THE COMMITTEE ON THE ADVANTAGES AND DISADVANTAGES OF THE VARIOUS LOCAL ANESTHETICS IN NOSE AND THROAT WORK *

Emil Mayer, M.D., New York, Chairman
Ross Hall Skillern, M.D., Philadelphia
Robert Sonnenschein, M. D., Chicago
Committee

(From The Laryngoscope, July, 1920, p. 443)

At the meeting of the Section on Laryngology and Otology of the American Medical Association, held in June, 1919, the following letter was received:

"The Council on Pharmacy and Chemistry, at its recent meeting discussed the deficiency of published data as to the special advantages and disadvantages of the various local anesthetics in nose and throat work. As chairman of the Committee on Therapeutic Research, I was commissioned to suggest to you that your section appoint a special committee to study and report on this subject. Presumably this committee would sift the available data and decide what further investigations would be necessary to clarify the subject.

"If we are not mistaken in our estimate of the importance of the subject, I feel assured that you will act on these suggestions. Should your committee need any assistance, financial or otherwise, our committee will be ready to cooperate to the extent of its ability.

"Very truly yours,

"Torald Sollmann."

Your committee was duly appointed. By a strange but happy fortuitous circumstance, we have the honor of presenting this report in New Orleans, the home of Prof. Rudolph Matas, who has done more to establish the value of local anesthesia than any other surgeon in the United States, to whom we are glad to pay our respectful homage for his splendid work in this direction, for his scientific attainments, and for the incalculable benefit he has thus given to suffering humanity.

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* An abstract of this report was published in J. A. M. A. **75**: 315 (July 31) 1920.

No work of this kind has been undertaken before by any national society, and your committee found itself confronted by a most arduous task; the literature alone on this subject is simply stupendous.

Owing to war conditions, many of the local anesthetics were not at hand, and hence the investigation was limited to those that were.

Realizing the vast importance of a study of this subject, the following plan was arranged as our objective:

To compare local anesthesia in its effects with general.

To note disadvantages following the use of any local anesthetic.

To check laboratory data by clinical data.

To ascertain what clinical evidence the literature affords in support of the use of the several local anesthetics in different conditions and of concentrated solutions rather than dilute in any case.

To determine what cases show idiosyncrasy with a view to determine causes.

To ascertain the relative merits of cocain and the synthetic products.

To determine if hemorrhage during or after operation is greater under local than under general anesthesia.

To study carefully the toxicity, noting especially injuries to the mucous membrane as factors in the latter conditions.

To note the effects of the previous administration of morphin in reducing dangers of toxicity or in adding to the comfort of the patient.

Finally, to make such recommendations as may be deemed advisable.

This tentative plan received the cordial endorsement of the entire committee, and of the chairman of the Committee on Therapeutic Research.

To Dr. Sonnenschein was given the task of abstracting the literature, to Dr. Skillern that of assembling the clinical data, and the animal experimentation suggested was kindly undertaken by Prof. R. A. Hatcher, of the Cornell University Medical College.

Not a single synthetic preparation that has been thus far presented for our use as a local aneshtetic is nontoxic. This does not deter us in its use, but it will surely put us on guard and free us from the belief that any one is absolutely free from danger.

ADVANTAGES OF LOCAL ANESTHESIA

Until 1914, only monographs on the various local anesthetics had been published. The entire subject of local and regional anesthesia was first presented in that year in a large volume by Dr. Carroll W. Allen.[1]

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[1] **Allen, C. W.: Local and Regional Anesthesia, 1918.**

Matas, in his introduction to Allen's book, says:

> Great transformations have taken place in our method of narcosis. Chloroform, which for half a century had reigned supreme as the autocrat of the operating theater, has been practically banished from the clinics of the South, its last stronghold.
>
> The problem of shock, secondary nausea and vomiting, the pulmonary complications, embolism and thrombosis, and above all the degenerative auto-intoxication following the action of these somatic poisons on the eliminating and other organs, still remain to be reckoned with.
>
> On the other hand, the synthetic chemist and pharmacologist have not been idle, and their untiring and brilliant efforts to find substitutes for the dangerous and costly cocain, have given us a succession of the remarkable synthetic products to be themselves displaced in turn by what now appears to be the nearest approach to the ideal local analgesic, novocain.
>
> The art of local and regional anesthesia is still young; it is still in process of development, still offers many difficult problems that await solution.

Allen,[1] calling attention to the fact that chloroform was discovered in 1831, ether in 1842, cocain in 1884, says:

> Had local anesthesia been discovered first, general anesthesia might now be struggling to replace it from its coveted pedestal, and it is not to be doubted but that local anesthesia would have reached a much higher plane of development, for in all operations suited to its use, general anesthesia cannot compare with it, in safety and comfort. The survival or failure of any method advocated for practical daily use must rest entirely on the clinical results obtained. The prime object of all surgery, as well as all medicine, is the relief of suffering, and the prolongation of life; those measures which obtain these ends with the least disturbance to the patient and the least suffering must ultimately prevail to the exclusion of all harsher and less agreeable.

In the hospital service of Dr. Matas and Dr. Allen, fully 55 per cent. to 65 per cent. of the major operations are performed solely by peripheral anesthesia.

Shambaugh,[2] in an editorial comment, says: "To depart from local anesthesia in nose and throat operations is a step in the wrong direction."

Thompson,[3] in writing of local anesthesia for tonsillectomy, says: "For complete removal in children, only general anesthesia should be considered, but this is seldom necessary in adults."

Mosher,[4] writing of the activities of the Section of Oto-Laryngology in the late war, says that most tonsillectomies are done under local anesthesia. Many conversions occurred to the use of local anesthesia, and the author implies his own partial conversion. He had expected deaths to be reported from postoperative hemorrhages, when done under local anesthesia, but none had been.

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2. Shambaugh, G. E. Practical Medicine Series 208, 1919.
3. Thompson, J. A.: Ohio State M. J. **14**: 469, 1917.
4. Mosher, H. P.: Mil. Surgeon **43**: 631, 1918.

Dowman[5] states that Thomas and Cushing reported the removal of a brain cyst under cocain anesthesia after four previous operations under general anesthesia had been abandoned on account of excessive hemorrhage due, presumably, to the fulness of the vessels produced by the anesthesia. They say:

> Although the undertaking was premeditated, in consequence of our previous unfortunate experience in administering general narcosis to this patient, we must confess to surprise at its successful accomplishment. Contrary to all expectations the dura proved to be insensitive to such manipulations as were necessary to open it freely. Only when it was put under tension or displacement was any discomfort occasioned, otherwise it seemed to be absolutely free from sensitivity.

The more general application, however, in the use of local anesthesia in operations for cranial and brain injuries as the result of war wounds was urged by Colonel Gray of the British army in 1917; also in developing the technic, which was responsible for the reduction of the operative mortality from 60 or 65 per cent. to less than 30 per cent. In head injuries with dural penetration, Cushing adopted the routine use of local anesthesia as one of the important steps in the operation.

In a very intensive experience as chief of a neurosurgical team working in the forward area during the St. Mihiel and Argonne offensives, Dowman became thoroughly converted to the use of local anesthesia in brain and spinal cord operations. The advantages were so numerous and the disadvantages were so few that he could see no reason why the practice should not be adopted more generally in operations for neurosurgical conditions.

Since returning to civil practice the writer has used local anesthesia as a routine practice in all neurosurgical operations, excepting those on children and on extremely nervous adults whose cooperation could not be relied on.

RÉSUMÉ

Thus presenting the views of the general surgeon, together with the opinions expressed by those operating in nose and throat conditions taken from most recent literature, we respectfully submit the advantages of local anesthesia in nose and throat operations.

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[5] Dowman, C. F.: Local Anesthesia in Neurosurgery, J. A. M. A. **74**: 383 (Feb. 7) 1920.

DISADVANTAGES

The disadvantages are claimed to be: Difficulty of control of bleeding at the time of operation; tendency to postoperative hemorrhage, greater than under general anesthesia; difficulty of sterilization; edema and sloughing; and greatest of all, toxicity.

A careful investigation among many American operators who favor local anesthesia in nose and throat work, and they are by far in the great majority, fails to elicit any unusual difficulty from bleeding at the time of operation, nor any greater frequency of postoperative hemorrhage after local anesthesia than after general. The danger of bleeding following a tonsil enucleation in an adult whose tonsil has already become fibrous is fully considered, and the careful operator bearing this in mind makes this a hospital operation, keeping the patient in bed three to four days thereafter, thus avoiding possible hemorrhage, and having prompt help on hand if needed.

STERILIZATION

There seems to be no doubt but that occasionally epinephrin loses its ischemic effect on boiling, and should therefore be always tested as to its activity.

Ersner[6] states that novocain is difficult to sterilize.

MacNaughton-Jones[7] has presented an article on the sterilization of local anesthetics which consists in preparing a solution of sodium chlorid together with the anesthetic or anesthetics and epinephrin chlorid, each constituent being present in such quantity that a dilution with many times the volume of water will give a solution containing the requisite percentage of each. The preparation is placed in a sealed vial or ampule and kept until required. Sterile water is added immediately before injection.

He prepares a concentrated solution of novocain as follows:

	Gm. or C.c.
Novocain	3·25
Sodium chlorid	3·25
Epinephrin solution (1: 1,000)	8
Water ....q. s. ad	25

This solution, when diluted with forty times its bulk of water, contains 3.25 parts per thousand of both novocain and sodium chlorid, gives perfect anesthesia, and has an osmotic pressure approximately equal to a four per thousand (0.4 per cent.) solution of the latter salt.

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[6] Ersner, M. S.: New York M. J. **105**: 21, 1917.
[7] MacNaughton-Jones, M.: Lancet **1**: 904, 1918.

In conversation, Dr. William A. Heckard, a dental surgeon, stated that he had made use of an apparatus for the *triple* distillation of water in which novocain was to be dissolved, and that it had been claimed that this added to its effectiveness. The apparatus had been in working order in the New York Base Hospital No. 5, where a large amount of plastic surgery of the face had been most successfully done. He also gave the name of the originator to whom your chairman wrote, asking him for any published data as also for any reasons he might give for the *triple* distillation. Although this was asked for in the name of the committee and the American Medical Association, the courtesy of a reply was not vouchsafed.

EDEMA AND SLOUGHING

The only local anesthetic which produces edema and sloughing is quinin and urea hydrochlorid. So many statements were found in the literature that this anesthetic had been abandoned in other fields of medicine, because of edema and sloughing, that writers who had presented favorable reports on this drug in nose and throat operations were communicated with by your committee. One writer, who had recorded 390 cases of tonsillectomies extolling this anesthetic which he had used for four years and is still so recorded, now states that he has not used it in two years, although no publication has been made retracting the former endorsement.

Still another writer, who stated that quinin-urea came nearest the ideal local, now says that he has ceased using it.

As far as nose and throat operations are concerned, this drug has practically gone into "innocuous desuetude."

Crile is recorded in favor of this drug, although C. W. Allen says that he looks forward to the time when the former will abandon this agent.

It is but fair to state that a recent writer extols this drug in the operative treatment of disease of the rectum, and in a personal communication states that he has not changed his views.

It is to be regretted that this effect obtains, because the anesthesia at the time of the operation is excellent and the recovery of the patient seemed to be much smoother with it because the anesthesia remained for several days.

IDIOSYNCRASY

We have sought most earnestly for anything that might show some reference to idiosyncrasy, and have one such reference.

Tobias and Kroner[8] report a case of cocain poisoning:

> Female, single, aged 43, had been treated for years for tachycardia, excessive perspiration, dysentery. A tooth was extracted; 1 c.c. of a 1 per cent. solution of cocain was used. Soon after injection, twitching of the left facial nerve, both arms and legs. Patient went home with her sister; two hours after injection, pupils dilated, reacted to light, respirations rapid, as also heart action. Complete paraplegia both arms and both legs, of a spastic character. Reflexes of arms and legs much decreased. Patellar and foot clonus. Complete anesthesia of mouth and pharynx. Speech interfered with, also deglutition. Two hours later, arms and legs better, five hours later, again complete paraplegia, face resembling tetanus, lower jaw and facial reflexes greatly increased. Pulse soft and rapid. Two weeks of illness with eventual recovery.

The writers state that while this case shows many of the stigmata of hysteria, these surely could be excluded. Such symptoms could not generally rise in a healthy organism as long as the internal secretory glands kept the normal balance. Should these be interefered with, the poison could easily produce its ill effects without hundrance.

In this case there was a preexisting neurosis of the sympathetic, tachycardia, excessive perspiration and tendency to diarrhea. The small dose of the drug ordinarily nontoxic was sufficient to upset the feeble balance of so many years and this, they suggest, may help to explain the term "idiosyncrasy."

TOXICITY

This chapter is the most important one in the discussion of disadvantages of local anesthesia, and merits most careful study and investigation.

We can conceive of nothing more distressing than a death occurring when least expected, within two or three minutes after the injection of a local anesthetic for an operation that is done in the largest number of instances to relieve conditions that may add to the comfort of the patient, thus prolonging life, but not immediately necessary, to which the patient submits at our earnest advice.

It is only by a study of these fatalities that we may hope to obtain a fuller knowledge of the contributing factors, and to reach conclusions that may tend to prevent their occurrence.

Because so little is known of this subject and because of its vital interest, our search has been thorough both into recorded and unrecorded cases.

The toxic effects may range from mild symptoms, grave and threatening ones, to sudden death.

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[8] Tobias, E., and Kroner, K.: Berl. klin. Wchnschr. **55**: 162-164, 1918.

COCAIN

Among recent deaths recorded are: Death following the injection of cocain, amount not specified, for dental extraction mentioned in the *British Dental Journal* **119**:198, 1918.

An editorial in the same journal for the same year, page 352, cites two recent cases of cocain poisoning with sudden death.

Other cases of poisoning by cocain will be presented later in this report.

PROCAIN

In the editorial above referred to, Fischer[9] is mentioned as stating that in a practice comprising over 15,000 cases, he has not had a single serious case of intoxication after injection of novocain, while he will ever remember the grave accidents after cocain injection.

RECENT DEATHS AFTER USE OF NOVOCAIN

Wright[10] reports a case of death following the intraspinal injection of 1.1 grain of procain. The anesthesia was complete. The prostatectomy finished, the patient became nauseated and pale with pin-point pupils, persistent vomiting, thready pulse, pupils contracted for twenty-eight hours, and death occurred.

Shoemaker[11] reports a death after subcutaneous injection of not more than 2.5 c.c. of 5 per cent. novocain. Necropsy did not explain death.

Brutt[12] reports a case of sudden death following the use of procain. Altogether 0.7 c.c. was used.

Female, aged 29, total deafness since the fourth year, result of glandular disease. There was also disturbance of speech function. Fourteen years previously operated for goiter, well for years thereafter. Has noticed swelling of the neck for several months; and for several weeks dyspnea on great exertion. No palpitation, no nerve disturbance, no other signs of Basedow, no other goiters in family. Well built, deaf, speech hard to understand; skin very dry, scaly; face puffy without edema. Neck materially large (40 cm.) as a result of left-sided enlargement of thyroid (rather soft — no fluctuation, mild pulsation). On the right, scar of old operation, no enlarged thymus. A number of large veins extend down from the lower end of right half of thyroid; heart and lungs normal. Diagnosis: Recurrent struma, probable form of Basedow's. It was decided to excise old cicatrix and resect the remainder of the thyroid. Eight days under observation, shortness of breath only signs, on great exertion. The day before operation and on the same day. Digitalisatum by mouth given (amount not stated). Three-quarters of an hour before local anesthesia, scopolamin, 0.0005, morphin, 0.01 injection, and then followed hardly 140 c.c. of 0.5 per cent. solution of novocain. When 30-40 c.c. had been used, patient became pale; as this is not unusual they continued. There was no condition of fear on the patient's part. Five minutes after the local anesthesia was given, the patient lying quietly all the time, the pulse suddenly ceased and also the respirations; face became pale and bluish, pupils widely dilated. Artificial respirations. Heart massage. Camphor injections. Oxygen inhalations. Some return of normal respiration. Superficial respirations after long pauses. Failing to resuscitate, cardiac cavity was opened and direct massage used. The first strong compression of the heart restored for a short time fresh red facial color and pulse could always be felt, no spontaneous heart contraction. Massage kept up for half hour with intravenous injection of 10 per cent. oxygenated salt solution to no effect.

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[9] Fischer: Brit. Dent. **114**: 352, 1918.
[10] Wright, F. R.: Journal-Lancet **28**: 714, 1918.
[11] Shoemaker: Surg., Gynec. & Obst., May, 1919, p. 528.
[12] Brutt, H.: Deutsch. med. Wchnschr. **44**: 577, 1918.

Necropsy: Neither status lymphaticus nor hypoplasia of the chromafin system nor any other cause for sudden death found. The small dose of scopolamin and novocain (with the usual amount of epinephrin added) could only be dangerous if some unexplained susceptibility exists.

The writer quotes Nettel on a toxicity following novocain and asks: "Did the injection get into a large vein?" Many punctures were made, so even if a vein had been entered, it would be with a small dose. *He can find nowhere in the literature a suggestion that danger exists from intravenous injection.* (He might study the work of American Pharmacologists.)

Death here was due to the heart, evidently an abnormal susceptibility to the drugs used on an unusually bad heart. The lesson to be learned is that the utmost care must be used with this combination of drugs in struma.

(From the Surgical Division of the Eppendorf Hospital, Hamburg.)

TOXIC EFFECTS—NONFATAL

Calle-Cottin and Demole[13] record a case of septic meningitis following intraspinal injection of novocain.

Four hours after the injection of 0.08 novocain, symptoms of meningitis appeared, reaching their height the next day; cerebrospinal fluid was cloudy, contained many polynuclears but no micro-organisms. Symptoms decreased on the third day, patient was well on the fifth. The solution was freshly prepared, asceptic precautions carefully carried out, nor did the symptoms show infection. They ask was this due to an istonic solution? They believe that there was a special idiosyncrasy.

Vongaza[14] injected 2 per cent. novocain several weeks old prior to teeth extraction; severe edema of face followed, pronounced hemorrhage from major palatine artery, subsequent palatal perforation. The author feels that bacteria must have grown profusely in the solution of novocain and their toxin produced tissue narcosis.

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[13] Calle-Cottin, E., and Demole, M. V.: Rev. neurol. **24**: 51, 1917.
[14] Vongaza: Abstr. in Laryngoscope **24**: 412, 1914.

Giffin and Gundrum[15] report a case of novocain poisoning where 1/12 grain of the drug was injected peridentally combined with 1/800 grain epinephrin. This patient recovered. Inquiry among a wide circle of friends showed that none of these had ever seen alarming symptoms occur after procain.

Flory,[16] in six out of 100 patients, found that there was some kidney irritation after relatively small doses. These may well be due to causes other than procain.

Achard[17] says that in the largest number, elimination of novocain, as shown by urinalysis, is between two and three hours, practically finished in the sixth; exceptionally it is slower, with the greatest intensity in the second hour. Where the elimination is slow, it is usually accompanied by violent headache.

DEATH FROM APOTHESIN

Jay Ireland[18] reports a case of death following spinal anesthesia after 3 grains of apothesin were introduced by the French method into the spinal canal (after mixing with spinal fluid) by gravity with the patient in a recumbent position.

> He was placed in the sitting position, but became cyanotic. Herniotomy was performed. Four hours later the systolic blood pressure was 98, the diastolic 62. Pulse was 60 and was very weak. Three days later, January 22, he was very incoherent and cyanotic, mumbled frequently and had to be restrained. January 23: More stuporous and involuntaries occurred. These continued till death. January 24: The right arm was flaccid and Babinski, Oppenheim, Gordon and Chaddock signs were positive on the right. He moved his lips, but made no audible sound. Motor aphasia was present. January 28: Left parotid gland was swollen. January 29: Right facial paralysis developed. February 1: Slight increased fremitus with crepitant râles, present in the right lower lobe posteriorly. Temperature was 102 F., respiration 32; right pupil was larger than the left; fundi normal. Patient died about six hours after lung signs began.

Death was due to cerebral hemorrhage; whether this hemorrhage was due to toxic effects of the apothesin on the cerebral vessels cannot be definitely stated. The fact that the blood pressure was lowered by spinal anesthetics would seem to indicate that it was not due to the usual cause of cerebral hemorrhage, i. e., high blood pressure with arteriosclerosis.

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[15] Giffin, R. B., and Gundrum, F. F.: California State M. J. **12:** 415, 1914.

[16] Flory, P. L.: Deutsch. Ztschr. f. Chir. **144:** 217, 1918.

[17] Achard, H.: Progr. méd., Series 3, **24:** 70, 1919.

[18] Ireland, Jay: Death Following Spinal Anesthesia, J. A. M. A. **74:** 19 (Jan. 3) 1920.

Hamilton[19] mentions epinephrin as an adjunct to and an antidote for apothesin. The simultaneous administration of epinephrin with apothesin, as with cocain and novocain, is not for any purpose other than to localize and intensify the anesthetic action, since its stimulating action on the heart and vessels is obtained only by intravenous injection; but the antidotal effect is indirectly affected even from the subcutaneous administration, because epinephrin prevents general absorption of the anesthetic, and by intensifying the action of apothesin, makes large doses unnecessary.

The value of epinephrin, therefore, for both purposes to limit absorption and to sustain the heart, is due to its characteristic properties of constricting the blood vessels and acting as a powerful cardiac tonic.

Apothesin is a local anesthetic which is materially less toxic than cocain, and is entirely free from habit-forming effects.

BETA-EUCAIN

Orr[20] found in the literature six severe cases of intoxication reported from the use of beta-eucain. For a Bassini herniotomy, 0.25 per cent. solution, 3.4 grains given, but leakage was considerable. Patient died. There was some doubt as to contributing cause.

BENZYL ALCOHOL

Macht[21] finds this drug to have lower toxicity than the cocain group, and as good anesthetic properties. It is quickly excreted, easily sterilized, cheap, and satisfactory clinically.

STOVAIN

Smith and Hatcher[22] state that Munchmeyer, in 1,189 cases of spinal anesthesia, found not more than 70 per cent. free from after or by effects. One fatality, seven transient paralyses. Madden reports three fatalities. Hardoin, sixteen fatalities. Pharmacologic experiments show stovain distinctly but slightly more toxic than novocain similarly administered, and recovery from it is not so prompt.

PROCAIN

McMechan[23] says that procain is one-fifth to one-seventh as toxic as cocain. In subjects of low blood pressure or cardiac disease, procain should be used with caution on account of its depressing action. Large doses in susceptible individuals may produce tonic and clonic spasm, agitation and accelerated shallow breathing. The addition of alkali increases very noticeably the efficiency of procain and other allied analgesics, because the free anesthetic bases penetrate more readily than do the salts, but this does not apply to intracutaneous injection. Addition of sodium bicarbonate may be of use for intraneural or subdural, but is definitely useful for surface anesthesia.

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[19] Hamilton, H. C.: Surg., Gynec. & Obst. **29**: 92, 1919.

[20] Orr, T. C.: A Case of Beta-Eucain Poisoning, J. A. M. A. **66**: 1857 (June 10) 1916.

[21] Macht, D. I.: J. Pharmacol. & Exper. Therap. **11**: 263, 1918.

[22] Smith and Hatcher: J. Pharmacol. & Exper. Therap. **9**: 231, 1917.

[23] McMechan, F. H.: Clin. Med. **26**: 105, 1919.

NIRVANIN

No reports are at hand regarding this drug, though its toxicity is exceedingly low.

NEW LOCAL ANESTHETICS (ALLOCAIN)

Kubota[24] reports that Nagayoshi Nagai isolated an alkaloid ephedrin from a Chinese drug, *Ephedra vulgaria* Rich, and afterward obtained a synthetic alkaloid mydratin closely related to ephedrin. These alkaloids have a similar constitution to epinephrin, and it is claimed that their pharmacologic action resembles qualitatively that of epinephrin.

By introducing into mydratin monethyl, diethyl and benzoyl groups synthetized secondary local anesthetics, Allocain S. (soluble) Allocain A. (insoluble).

The object of the author was to obtain a body possessing the local anesthetic properties of cocain, as also vasoconstricting action like that of epinephrin. It is claimed for Allocain S. that among other properties it inhibits the growth of streptococci and staphylococci. It has been tried on several hundred cases of operations with success.

CLINICAL INVESTIGATION

In the belief that all fatal cases are not reported, the Council on Pharmacy and Chemistry made an attempt some time ago to secure some data on the toxic effects of local anesthetics through advertisement in more than thirty medical journals, receiving but two replies. Your committee had a similar experience through an announcement in THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION, and had but one reply and that reply "had nothing to do with the case." Consequently, we prepared a questionnaire seeking information directly from fellow members of this Association, and 85 per cent. of these answered. While this large number of responses is eminently gratifying, your chairman regrets that even so small a percentage of the members of this section could be found who failed to reply to a courteous query on a matter of such vital importance to the profession, as also to themselves, and to their own committee. We deem it important that attention should be called to this, as we believe that future investigation should result in 100 per cent. answers.

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[24] Kubota, S.: J. Pharmacol. & Exper. Therap. **12**: 61, 1919.

The result of this questionnaire has been exceedingly interesting and valuable. We have not only been able to secure reports as to the anesthetic used, its dosage, etc., but have also many reports as to toxic effects and a record of twenty deaths, most of them not previously recorded.

In dwelling on the question of toxicity in detail, we have done so because these cases are not all recorded, and only by careful examination can we hope to ascertain their causation and prevent their occurrence.

It must be understood that there is no intent to create the impression that fatalities are at all frequent; the percentage must be remarkably low, and the dangers of toxicity will compare most favorably with those of any general anesthetic.

REPLIES TO QUESTIONNAIRE

To 150 representative laryngologists of the United States and Canada, the following questionnaire was submitted:

1. What method of local anesthesia do you use for operations on the nose?
2. On the throat?
3. Do you use an anodyne first? If so, what and in what doses?
4. What toxic effects have come under your notice?
5. Do you know of any fatalities? Give particulars.

Answers were received from about 100; a few answered briefly, others quite fully and some in considerable detail. The following report is based on these and consists of a digest rather than a detailed account; the tabulated form will be found at the end of the report.

1. What method of local anesthesia do you use for operations on the nose?

The preference for a strong solution of cocain (10 per cent. or above) applied with cotton applicator or pledgets was marked, about 80 per cent. advocating its use. Powdered cocain, cocain mud and saturated solutions were used by twenty-six, while very weak solutions (from 1 to 4 per cent.) found favor among four. Injections of novocain, from 0.25 to 2.5 per cent.; cocain, 0.5 per cent.; apothesin, 1 per cent.

and procain, 0.5 per cent., were mentioned by twenty-two, but most of these used this means to reinforce the applications to the mucosa rather than as an isolated method, although one would gather that several place great stress on these injections as the sole means employed. One speaks of nerve blocking with cocain. Serials were used in five instances:

(*a*) 4 per cent. cocain with epinephrin followed by powdered cocain.

(*b*) 5 per cent. cocain with epinephrin followed by 10 per cent. cocain and epinephrin.

(*c*) 10 per cent. cocain with epinephrin followed by powdered cocain.

(*d*) 20 per cent. cocain with epinephrin followed by 0.5 per cent. procain injected.

(*e*) 1: 5,000 epinephrin chlorid followed by 10 per cent. cocain.

2. What method of local anesthesia do you use for operations on the throat?

The divergence of views regarding drugs and dosage was even more marked here than in the preceding question. Novocain in varying strengths was advocated by forty-four, 0.5 to 1 per cent. solution in epinephrin being the most popular, although the degree scaled from 0.5 to 5 per cent. Cocain from 0.1 to 4 per cent. mostly with epinephrin was used by seventeen, and apothesin from 0.5 to 5 per cent. by fifteen. Procain (American novocain) was used by three, and anocain by four, while urea-quinin had but one advocate. For the larynx, the greatest unanimity was observed in 10 to 20 per cent. cocain.

3. Do you use an anodyne first? If so, what and in what doses?

The answers left one in some doubt whether the anodyne was used before nose or throat operations or both. One would gather, however, that for the most part anodynes were administered according to condition, i.e., if they appeared to be indicated, the patient being nervous and apprehensive, they were given, otherwise not. Certain operators made their administration previous to operation a routine procedure, but these were in the great minority, others prescribed them p. r. n., as stated above; and not an inconsiderable number appeared to dispense with anodynes altogether, while one or two expressed themselves forcibly against their use. Morphin and atropin were the overwhelming favorites, both for nose and throat work. Scopalamin (hyoscin) was used in about six instances. Codein, heroin, sodium bromid and others were only occasionally advocated.

4. What toxic effects have come under your notice?

NOSE

Paleness, nausea, giddiness, symptoms of faintness, profuse perspiration (Butler).

Sudden paleness, rapid pulse, faintness which disappeared on keeping patient's head in lowered position for a few moments (Skillern).

Psychic only (Mackenzie).

None since waiting twenty minutes (Ridpath).

Slight nausea, sweating, rarely vomiting (Canfield).

Occasional faintness, stiffness of forearms and fingers, thumbs drawn across palm, increased pulse and respiration (Chamberlin).

Typical poisoning from cocain in flakes (Beck).

Few instances from cocain, faintness, oppressed breathing, weak pulse, numbness in arms and legs, flexion of thumbs and hysterical symptoms (Dennis).

Occasional cases of syncope (Hubbard).

Collapse, slow, thready, weak, irregular pulse, dyspnea, pallor, lividity of mucous membrane (Friedberg).

Rapid, feeble, nervous excitement and alarm, faintness (Crosby Greene).

Apothesin produced mild symptoms similar to cocain poisoning in two cases (Lockard).

Cocain, 2 per cent., preliminary to anesthesia for irrigation of antrum, complete collapse, Cheyne-Stokes breathng, deep coma for four hours, final recovery (Lockard).

Occasional fainting which has not recurred since using the recumbent position for operating (Haskin).

Faintness and occasional nausea with cold sweat (Thurber).

Slight constriction to breathing, increased respiration, dilated pupils, nervousness and excitability (Levy).

Profuse sweating and feeling of faintness during first few minutes (Barnes).

Tendency to faint in some cases (Craig).

Occasional dizziness, feeling of faintness and slight nausea (Holliday).

Great interference with respiration, cardiac failure, patient nearly died from injection of 0.5 per cent. anocain.

Transitory pallor and faintness (Mackenzie).

In several hundred septum operations, about 60 per cent. felt faint (Alexander).

Marked depression even with 5 per cent. solution cocain (Wishart).

THROAT

Effects from 0.5 per cent. solution cocain (Eves).

Effects from apothesin, 1 per cent., neurotic woman, pulse became very feeble, convulsion, unconscious two hours (Lewis).

Effects from novocain, 1 per cent., sudden pallor, weakness, pulse feeble, semicomatose one hour (Lewis).

Occasional sweating with nausea when stronger epinephrin was used, headaches, sometimes severe (Canfield).

Mild cases from novocain (Dean).

Acute delirium following removal of papilloma of larynx (Chamberlin).

Fainting, nausea, rapid pulse, clammy skin, largely obviated by keeping patient reclining or semireclining during cocainization and operation (Roberts).

Patients sometimes get talkative and a few a little depressed (Dudley).

Some pallor and faintness at times during tonsillectomy (Hastings).

Cocain poisoning, case cauterization of larynx and case bronchoscopy (Guggenheim).

Apothesin injection, 2 per cent., complete collapse, unconsciousness, intermittent contractions of all muscles, Cheyne-Stokes breathing for one hour (Lockard).

Tendency to syncope in a few cases (Royce).

Paleness, increased heart action, three cases became unconscious for two minutes, two cases vomited (Campbell).

Many believe that fear and apprehension play a large rôle in the appearance of so-called toxic symptoms (faintness, trembling, hot and cold flushes, increased pulse, etc.); others state that waiting twenty minutes before commencing the operation will avoid these symptoms. It would appear that anemia of the brain played an important rôle, as lowering the head invariably brings quick relief. An excessively hot operating room will strongly predispose to bring out these symptoms.

5. Do you know of any fatalities? Give particulars.

A. Weak solution procain and epinephrin into septum of nose.
B. Injection 10 per cent. solution cocain in septum (mistake for 1 per cent. solution).

THROAT

1. Novocain injected.
2. 0.5 per cent. cocain in epinephrin, 1: 10,000.
3. 0.5 per cent. solution cocain (two deaths).
4. 1 per cent. cocain in epinephrin, 1: 1,000, equal parts.
5. Injection 5 per cent. solution cocain (instead of swabbing).
6. 0.75 per cent. novocain, injection with 1: 30,000 epinephrin into peritonsillar tissue.
7. 10 per cent. cocain injected (mistake for 1 per cent. procain solution).
8. 20 per cent. solution cocain in larynx.
9. 10 per cent. cocain injected around tonsil (mistake for 0.1 per cent. solution cocain).
10. Cocain and epinephrin injection, strength not stated.
11. 0.2 per cent. cocain in epinephrin injected, death in one hour.
12. Injection 0.25 gr. cocain into anterior pillar, death in four minutes.
13. Injection 0.5 per cent. novocain.
14. Injection 2 per cent. cocain with equal parts epinephrin full strength.
15. Cocain applied to throat and epiglottis.
16. 0.5 per cent. novocain injected around tonsil.
17. 20 per cent. solution was injected instead of 0.5 per cent. cocain.
18. 0.5 per cent. solution novocain injected after painting with 10 per cent. cocain.

1. No further details. No amount stated.

2. Large fat girl of about 20 for tonsillectomy. About three drops of solution said to have been injected, immediate death; 0.5 per cent. cocain in epinephrin 1 : 10,000.

3. (*a*) A girl of about 20 with severe endocarditis. Injection of tonsil, immediate death. Necropsy: large vegetations in heart, interference with valve action. Heart stopped in systole; 0.5 per cent. solution cocain—two deaths.

(*b*) Officer of 25, previously operated (submucous and ethmoid) without toxic effect. One tonsil injected, immediate death. Necropsy: marked enlargement of thymus with general glandular enlargement; 1.2 per cent. solution cocain.

4. Injection of 20 minims of equal parts of 1 per cent. cocain and epinephrin 1 : 1,000 for tonsillectomy. The patient became faint and died in less than three minutes.

5. No further details. Injection 5 per cent. solution cocain instead of swabbing.

6. Immediately following injection of less than 1 dram into peritonsillar tissue great apprehension, air hunger, convulsive movements succeeded by cessation of respiration and gradual cessation of pulse; 0.75 per cent. solution injection novocain 1 : 30,000 epinephrin into peritonsillar tissue.

7. No further details; 10 per cent. solution cocain injected, mistaken for 1 per cent. procain solution.

8. Twenty per cent. solution cocain in larynx. Patient died almost instantly after application.

9. Robust man about 43. After two syringefuls fell to floor unconscious and died in spasm in few minutes. (Mistake of druggist.) Ten per cent. cocain solution injected around tonsil, mistaken for 0.1 per cent. solution.

10. Patient man of about 28, had an attack of angina pectoris and died two hours after operation. Necropsy showed fatty degeneration of heart muscle. Cocain and epinephrin injected, strength not stated.

11. Patient received in all about 2 drams 0.2 per cent. cocain epinephrin injected, death in one hour.

12. No further details. Injection ¼ grain cocain in anterior pillar, death in four minutes.

13. Woman 30 years. Local application of cocain 10 per cent., followed by injection novocain 0.5 per cent. with 8 drops epinephrin to 4 drams. One dram injected in each tonsil. First tonsil removed when patient gasped and died.

14. Woman 21 years. Three syringefuls injected under the mucous membrane of each faucial pillar. Injection 2 per cent. cocain with equal parts epinephrin 1 : 1,000.

15. No further details. Cocain applied to throat and epiglottis.

16. Woman 25 years. Injection about 100 minims 0.5 per cent. novocain with 2 drops epinephrin chlorid. After about three minutes a slight convulsive movement involving head and neck was noticed, followed in a few seconds by one more severe attack including shoulders, chest and upper arms. Tightly closed jaws, dilated pupils, fixed gaze, wide palpebral fissures, stertorous breathing, moderate but soon marked cyanosis, passing of the violent convulsive seizures into a marked and general tremor. Cardiac action 120, complete respiratory paralysis in ten minutes. Cardiac action continued some time.

17. Injected into tonsil, the patient dying instantly. (Mistake of druggist.) Twenty per cent. solution cocain injected instead of 0.5 per cent.

18. Soldier, name unknown, aged about 23. Base Hospital 27, France. Patient not specially prepared; that is, he was given no cathartic night before and was allowed breakfast

morning of the operation. About 10 a. m. or one-half hour before operation, morphin sulphate, gr. $\frac{1}{6}$, was given hypodermically. Fifteen minutes before walking about 100 feet from one building to another, pharynx and fauces were swabbed with cocain 10 per cent. by the head nurse, who did this routinely. After patient entered the operating room, throat was again swabbed with cocain 10 per cent. After waiting a minute or two, injection of a 0.5 per cent. solution of novocain (French preparation) was started, patient in the sitting posture, and while this was being done patient was suddenly seized with what appeared to be an epileptic attack. Convulsive seizure lasted about one and one-half minutes, followed in a few seconds by another of shorter duration, which in turn was followed by six and finally death in about four to five minutes.

Patient received about 5 drams of a 0.5 per cent. solution novocain, or roughly $1\frac{1}{2}$ grs. Operation had not been started. After taking his seat in the operating room and before cocain was applied the second time, patient was quite pale, and the writer is inclined to think that the additional cocain and novocain served as the last straw, although it was the opinion of the committee that reviewed the case that novocain probably entered a vein. Solution out of same bottle was used on another patient a short time before. It is not known whether or not patient was an epileptic.

A review of these fatalities, twenty-one in all, two in nose operations, nineteen in throat operations, shows that fifteen occurred as the result of the administration of cocain, five from novocain and one from procain.

Of the cocain deaths, five were due to avoidable mistakes. The remainder died within a very short time, as a rule after the administration of the drug, three minutes being the usual amount of time.

Some of these cases have already been recorded, and there is no means at hand of ascertaining the proportion of deaths occurring as compared to the number of cases in which local anesthesia was used. As deaths have been recorded following rapid injections of epinephrin in 1 : 1,000 solution, we have carefully gone into this question, but find no evidence at hand that epinephrin in these cases was a causal factor. In many no epinephrin is mentioned—in others, weak dilutions.

It having been claimed, from very reliable sources, by most competent observers that the previous administration of morphin added greatly to the lasting effect of the local anesthetic, made the patients much more comfortable, requiring less of the local anesthetic, probably materially lessening its dangers, this subject was included in the questionnaire, and animal experimentation made to help elucidate it.

About three fourths of the replies received from clinicians indicated that morphin was used as occasion required, only a small minority using it invariably before operation. A few objected to it entirely, mainly on theoretical grounds. Those who have used it invariably have expressed themselves as agreeing entirely with the suggestion of Crile in his anoci-association, and it was ascertained from Professor Hatcher that he had made the following experiment:

"A cat was given a poisonous dose of chloral hydrate, replaced in its box and slept off its effects. Another was given the same dose and fastened to a board, when it promptly died." This would indicate that excitement might be an added danger, which morphin would tend to subdue.

Your committee appends herewith a brief résumé of recent pharmacologic studies on local anesthesia:

PHARMACOLOGIC STUDIES ON LOCAL ANESTHESIA

Recently Sollmann[25] has investigated several of the local anesthetics with reference to their relative anesthetic activity and the influence on this activity of the addition of alkalis, epinephrin and other agents. The results of these investigations are summarized in a paper published in The Journal of the American Medical Association.[26] His work shows that for intracutaneous anesthesia the hydrochlorids of cocain, procain (novocain), tropacocain and alypin are about the same inefficiency. Beta-eucain hydrochlorid is about half as efficient; quinin-urea hydrochlorid about one-fourth, and apothesin about one-eighth as efficient. When applied to the cornea, the hydrochlorids of cocain and holocain are about equally efficient; that of beta-eucain one-half; those of tropacocain, alypin and quinin-urea about one-fourth; apothesin, one-eighth, and procain (novocain) one-sixteenth as efficient as cocain. Slightly different relative efficiencies are obtained by other methods of testing, but none of these methods give results which are comparable to those which may be expected from the clinical use of the drugs in man.

By infiltration the action is induced almost immediately, but when applied to the surface, as to mucous membranes, tropacocain, alypin and quinin-urea were the most rapid; beta-eucain was intermediate; and cocain, procain and holocain were the slowest. For surface anesthesia (rabbit's cornea) the duration of anesthesic action was found to be from ten to fifteen minutes with cocain and tropacocain; from twenty to thirty minutes with alypin, beta-eucain and holocain; fifty minutes for procain, and sixty-five minutes for quinin-urea.

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[25] Sollmann, Torald : J. Pharmacol. & Exper. Therap. **10**: 379, 1917-1918; **11**: 1-9, 17, 69, 1918.

[26] Sollmann, Torald: The Comparative Efficiency of Local Anesthetics, J. A. M. A. **70**: 216 (Jan. 26) 1918.

Gros found that the addition of alkali to the local anesthetics increases their anesthetic efficiency as tested on the motor fibers of nerve trunks. Sollmann confirms this and finds that this increased activity also involves the action on the sensory fibers of nerve trunks and the action of the anesthetics when applied to the surface, as on the rabbit's cornea or the frog's skin. This increased activity, or potentiation, by alkali is due to the liberation of the anesthetic base which penetrates more readily than the salt. No such potentiation occurs when the anesthetic is used by infiltration, and the addition of alkali serves no useful purpose for infiltration anesthesia. The addition of an equal volume of 0.5 per cent. solution of sodium bicarbonate to a solution of cocain for surface anesthesia increases the activity of the cocain from one to two times; the activity of beta-eucain is increased two times; procain from two to four times; tropacocain and alypin four times. This effects a marked saving in the amount of anesthetic drug used.

The addition of epinephrin to the local anesthetics does not increase their anesthetic activity, but for infiltration it greatly prolongs their action by prevention of absorptive removal.

A 1 per cent. solution (isotonic solution) of potassium sulphate or chlorid has some anesthetic action, but too little to be of value alone. It is an advantage, however, to substitute such a solution for sodium chlorid in preparing solutions of the local anesthetics for infiltration anesthesia.

The pharmacology of the local anesthetics has also been investigated recently with special reference to the causes of intoxication, the elimination of the drugs from the animal body, and the possible measures of combating intoxication. The data are to be found in papers by Hatcher and Eggleston,[27] Smith and Hatcher,[28] Eggleston and Hatcher,[29] and Eggleston and Hatcher,[30] from which the following is abstracted.

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27. Hatcher, R. A., and Eggleston, C.: J. Pharmacol. & Exper. Therap. **8**: 385, 1916.

28. Smith, M. I., and Hatcher, R. A.: J. Pharmacol. & Exper. Therap. **9**: 231, 1917.

29. Eggleston, C., and Hatcher, R. A.: J. Pharmacol & Exper. Therap. **13**: 433, 1919.

30. Eggleston, Cary, and Hatcher, R. A.: The Pharmacology of the Local Anesthetics, J. A. M. A. **73**: 1256 (Oct. 25) 1919.

The symptoms of intoxication produced by the local anesthetics are closely similar in both man and the lower animals, especially the cat. The toxic phenomena produced by the different local anesthetics are also closely similar. The maximum toxicity of the local anesthetics can be determined on the cat by the rapid intravenous injection of concentrated solutions. It is further found that all of the local anesthetics are mutually and quantitatively synergistic so far as their fatal actions are concerned.

With slow or repeated intravenous injection, the cat can withstand several times the fatal vein dose of all of the local anesthetics excepting cocain and holocain. This is due to the rapid elimination of the local anesthetics.

With subcutaneous injection into the cat the toxicity of the local anesthetics depends on the ratio between their rates of absorption and of elimination. Thus, five or more than five times the minimal fatal vein dose of alypin, apothesin, beta-eucain, nirvanin, procain, stovain and tropacocain can be injected subcutaneously into the cat without causing death. Four, or less than four times the fatal vein doses of cocain and holocain, similarly injected prove fatal. If epinephrin be injected subcutaneously simultaneously with the local anesthetics, their toxicity is greatly reduced by delay in their absorption. This reduction of toxicity is much less marked in the cases of cocain and holocain than with others of the group, owing to their much slower essential elimination.

The various local anesthetics are absorbed from the mucous membranes of the nose and pharynx of .the cat no more rapidly than from the subcutaneous tissues, and the urethra, bladder and vagina resist the absorption of these drugs to a great extent.

The elimination of the local anesthetics in the cat is shown to be through their rapid destruction in the liver, and this takes place in the excised, perfused organ as well as in the liver of the intact animal.

Sever acute hemorrhage and narcosis by hydrated chloral are the only procedures by which the toxicity of the local anesthetics for cats could be increased, and the increased toxicity sems probably to be due to a diminution in their rate of destruction in the liver through an impairment of the circulation.

All of the local anesthetics are synergistic with epinephrin on the blood pressure in a manner analogous to cocain, when injected intravenously.

The use of artificial respiration, combined with stimulation of the heart by the intravenous injection of epinephrin, is capable of saving cats from death following the intravenous injection of as much as twice the average fatal vein dose of the local anesthetics. Similarly, the stimulation of the heart by the previous injection of ouabain permits the cat to recover from intravenous injections of nearly twice the average fatal dose of any of the local anesthetics, when the temporary paralysis of the respiratory center is combated by means of artificial respiration. The success of both of these measures rests on the rapid destruction of the anesthetics by the liver.

All of the local anesthetics kill the cat by immediate and simultaneous paralysis of the heart and the respiratory center, the action on each being independent of that on the other.

The relative toxicity of the local anesthetics cannot be stated in figures, since it depends on the mode of administration, the rate of absorption or entrance into the blood stream, and the rate of destruction in the liver.

The simultaneous employment of epinephrin with the local anesthetics for infiltration anesthesia is of value, not only in prolonging the anesthetic action, but in diminishing the danger of intoxication by delaying absorption. The danger of intoxication by the local anesthetics is more effectively reduced in the cases of all of the other local than in the cases of cocain and holocain, because these are less rapidly destroyed in the liver than are the others.

ANIMAL EXPERIMENTATION MADE FOR THIS INVESTIGATION

"Department of Pharmacology,
Cornell University Medical College,
New York, Dec. 13, 1919.

"Dear Dr. Mayer:

"I have performed a few experiments on dogs and rabbits, using procain, without, and with morphin and atropin, on dogs by intramuscular injection of small doses of procain (25 milligrams per kilo) and on rabbits by intravenous injection of the same amounts. They afford no evidence that these alkaloids have any marked influence on the toxicity of procain (or novocain), though morphin does certainly have some effect in modifying the severity of the convulsions, and probably on the rate of complete recovery following such large intravenous doses.

"I speak of this as a large intravenous dose, though it is the same dose per kilo as that used intramuscularly, in which it is in effect a relatively small dose, as it produces no perceptible effect when injected intramuscularly.

"Yours very truly,

"Robert A. Hatcher."

PROTOCOLS

Local Anesthetics: Procain with morphin and atropin: Dogs: Intramuscular. Dec. 17, 1919.

1. White and brown mongrel, male, wt., 4.46 kilos.

4: 46 p. m. Injected 25 milligrams per kilo intramuscularly, in 5 per cent. solution; there was no perceptible effect; animal remained normal.

2. Male, mongrel, brown, wt., 3.94 kilos.

4: 08 p. m. Injected intramuscularly 0.2 mg. atropin sulph. × kg. with 10 morph. sulph. × kg. Animal became somewhat depressed, showing usual effects of morphin, but no emesis; some salivation.

4: 50. Twenty-five mg. procain (novocain) × kg. in 5 per cent. solution intramuscularly; animal remained quiet, showing effects of morphin but none other.

9: 00 a. m. (following day). Animal appears quite normal.

3. Male, mongrel, white and black, wt., 3.14 kilos (somewhat emaciated).

4: 13 p. m. Injected 10 mg. morphin sulph. × kg. 2 per cent. solution intramuscularly; effects much as in preceding.

4: 52. Injected 25 mg. novocain (procain) 5 per cent. solution intramuscularly.

5: 15. There has been no perceptible effect (results as in preceding).

9: 00 a. m. (following day). Animal appears to be quite normal.

The foregoing would seem to indicate that morphin alone, and morphin with atropin in moderate doses, does not influence the action of procain when injected intramuscularly in such a way as to afford any explanation of accidents often observed when it is used therapeutically.

These experiments have no bearing on the question whether morphin alone, or with atropin, increases or diminishes the toxicity of procain for dogs, except so far as they indicate that morphin and atropin do not increase the toxicity after intramuscular injections to any extraordinary degree.

Local Anesthetics: Procain with morphin and atropin: Rabbits, intravenous. Atropin, 1-1,000; morphin, 1-50; procain, 1-20.

Dec. 18, 1919:

1. Brown male, 0.87 kilo.

9: 55 a. m. Injected into ear vein, 25 mg. procain in about five seconds. Convulsions in about thirty seconds; violent. Animal sat up after about seven minutes and appeared normal in fifteen minutes.

10: 43. Injected into ear vein 2 mg. morphin sulph. and 1 mg. atropin sulph. × kg.

Animal became greatly depressed at once, sitting with head to floor; easily aroused, but sinks down at once when undisturbed.

10: 47. Injected 25 mg. procain × kg. into ear vein. Mild convulsions almost at once; respiration became very shallow for period of several minutes and animal fully extended.

10: 53. Able to get up, but falls when attempting to hop about.

11: 40. Sitting up in cage, slightly depressed. Recovered gradually and completely in about three hours.[31]

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[31] Rabbit 1 had a large tumor over the forehead; but appeared normal otherwise. After having been apparently normal it was observed to be dying at 2: 45 p. m. and was found dead a little later.

2. Brown male, 1.14 kg.

10: 11 a. m. Injected 12 mg. morphin sulph. × kg. into ear vein. Effects as in preceding.

10: 17. Injected 30 mg. procain × kg. into ear vein. Feeble convulsions at once; respiration became very shallow; animal fully extended.

10: 26. Respiration slow and shallow; eyes half open; nearly fatal.

10: 32. Sits on belly; not fully extended as previously; recovery.

11: 02. Able to get up but sits quiet when undisturbed.

11: 45. Still flat on belly.

1: 20 p. m. Improving, but sits with head to floor of cage when undisturbed; recovered completely.

3. White and brown female, wt., 1.22 kg.

10: 30 a. m. Injected 2 mg. morphin sulph. and 1 mg. atropin sulph. × kg. into ear vein. Sits with head down to floor at once when released; easily aroused to move about.

10: 35. Injected 25 mg. procain × kg. into ear vein. There was no perceptible effect leading to an examination of the ear to determine whether the fluid had escaped from the vein; no edema could be discovered, but effects lead to belief that previous injections had caused some anomalous condition preventing the drug from entering the circulation at once, hence the following:

10: 57. Injected 25 mg. procain × kg. into ear vein with precautions to see that it entered circulation at once. Convulsions at once, but mild as in case of others after morphin.

11: 05. Still much depressed; does not turn over when placed on back with feet pointing upward in vrey awkward position.

11: 40. Sitting up in case but not so nearly normal as is No. 1 at this time. Gradual and complete recovery with return to normal in about three hours.

The foregoing indicate that morphin modifies the convulsions of large intravenous doses of procain lessening their severity, but that neither morphin alone, or with atropin, in the doses used, has any material influence on the toxicity of intravenous doses of nearly fatal doses of procain in the rabbit.

Local Anesthetics: Morphin, atropin, cocain: Cats; vein and subcut. Jan. 16 1920.

1. Male, gray striped; 3.16 kg.

11: 08 a. m. 10 mg. morph. sulph. × kg. 1-50. 1 mg. atrop. × kg. 1-1,000 subcut.

11: 28. Shows little effect of morphin (cf. No. 2).

11: 31. 45 mg. cocain HCl × kg. 1-20 (equivalent of three times fatal vein, injected subcutaneously).

11: 36. Becoming restless (morphin action).

11: 50. Mild general clonic convulsions.

1: 00 p. m. Frequent spasmodic twitches; quiet in intervals.

3: 29. Sudden violent general clonic convulsions.

3: 40. Condition closely resembles that of No. 2 at 11: 28.

4: 32. Convulsions as at 3: 29.

5: 02. Convulsions as at 3: 29.

6: 30. Almost incessant convulsive movements as though suddenly frightened since 5 p. m.

8: 00. Condition much as at 6: 30, but seizures less frequent. Typical morphin action in cat. Pupils are widely dilated; do not react to light (atropin).

January 17:

10:00 a. m. Depressed; fatigue from long continued convulsive condition; pupils widely dilated; still slightly hyperexcitable; markedly so in fact, but relatively slight as compared with previous condition. Animal used for another drug experiment.

2. Female, gray stripes, 3.38 kg.

January 16:

11:10 a. m. 10 mg. morph. sulph. × kg.; 1-50 subcuta.

11:28. Typical morphin action; very restless and almost convulsive.

11:33. 45 mg. cocain HCl × kg.; 1-20 subcut.

11:36. Frequent clonic convulsions.

11:50. Lies extended; frequent mild clonic jerks.

1:00 p. m. Lies quiet except for occasional clonic jerks.

3:30 Animal is dead; probably died about 3 p. m. Exact moment of death not observed.

Eggleston and Hatcher found that the cat survived. Cocain in doses slightly larger relatively than these, and this would seem to indicate that morphin alone increases the toxicity of cocain for the cat by subcutaneous injection, and that atropin lessens the effect of morphin, but the dose is at any rate so near the fatal (for cocain) and the cat that received the atropin with the morphin was so nearly fatally poisoned that one is not justified in attributing any marked effect either antagonistic or synergistic to the morphin and atropin, or morphin alone, with cocain.

Local Anesthetics: Procain after scopolamin: Rabbits: Vein, subcut.

Jan. 16, 1920: Procain 1-10; scopolamin 1-500.

1. Gray rabbit, wt., 1.76 kg.

3:13 p. m. 2 mg. scopolamin HBr (euscopol) 25 mg. procain × kg. ear vein; respiration became hurried at once, then animal was somewhat depressed for about two to three minutes with rapid and complete recovery.

3:25. Pupils are widely dilated; do not react to light.

2. Gray, wt., 1.23 kg.

3:18 p. m. 2 mg. scopolamin HBr (euscopol) × kg. intramuscularly.

3:21. 100 mg. procain × kg.; 1-10 subcutaneously.

3:25. Pupils dilated, not maximal; react slightly to light.

3:40. Lies fully extended; nose to floor.

5:05. Appears to be about normal.

Local Anesthetics: Procain after scopolamin: Cat, vein, subcut.

Jan. 16, 1920: Scopolamin HBr (euscopol), 1-500; procain 1-10.

1. Female, 1.95 kg. (phenol locally for operation).

2:23 p. m. 1 mg. scopolamin HBr; 25 mg. procain × kg. vein convulsions at once; respiration interrupted about fifteen seconds, followed by rapid recovery.

3:22. Pupils dilated widely; do not react to light.

5:05. Appears normal.

2. Female, 2.73 kg.

1:55 p. m. 2 mg. scopolamin HBr (euscopol) × kg. intramuscularly.

2:06 100 mg. procain × kg. 1-10, subcutaneously.

2:14. Emesis.

3:25. Pupils dilated, not maximal; react slightly to light.

5:05. Animal is somewhat hyperexcitable; otherwise appears nearly normal.

SECOND SERIES OF EXPERIMENTS

"Department of Pharmacology,
Cornell University Medical College,
New York, March 6, 1920.

"Dear Dr. Mayer:

"I enclose the protocols of several experiments in which six doses of procain were administered to rabbits (in addition to those already reported to you) and these confirm the observation made previously that the use of morphin causes the convulsions following the intravenous injection of procain to be either milder or of shorter duration. It is plain, however, that this effect results from the depression caused by the morphin, and that this depression is added to that of the procain with the result that recovery is slower and the danger of death is increased.

"In view of the fact that such large doses are used and the difference is so slight and not absolutely uniform, I believe that the observation has slight value. The results certainly do not point to the value of the use of morphin with the local anesthetics; on the other hand, I would not conclude from these results that morphin is necessarily contraindicated. The fact that morphin depresses the respiratory center, taken with the fact that the various local anesthetics also cause depression, and parlysis with large doses, of respiration certainly enjoins circumspection with reference to their simultaneous use, and I believe that a very definite need of morphin would alone justify its use previous to, or with, these local anesthetics.

"Yours sincerely,

"ROBERT A. HATCHER."

PROTOCOLS

Local Anesthetics: Procain and morphin: Rabbits:

March 5, 1920:

4. Brown rabbit, 2.33 kg.

11: 23 a. m. 30 mg. procain × kg. in 10 parts N. S. into ear vein at once. Animal released at once; fell on side. Moderate convulsions.

11: 27. Begins to raise head from floor (first signs of recovery).

11: 31. Sits up on belly, but head to floor.

11: 33. Begins to hop about; nearly normal.

11: 47. 12 mg. morphin sulphate × kg. in 50 parts N. S. into ear vein; when released the animal sits quiet with head to floor after about two minutes.

11: 52. 30 mg. procain × kg. as previously. Released at once; slight convulsion; then lies on side fully extended; convulsion was somewhat more severe than previously, but briefer. Animal assumed unusual position with hind leg pointing upward, remaining in this position about an hour.

3: 30 p. m. Sits with belly flat to floor and head down when not disturbed, but able to hop about; returned to cage.

12: 02. Respirations shallow; 20 per minute.

5. White rabbit, 2.37 kg.

11: 21 a m. 2 mg. morph. sulph. × kg. in 50 parts N. S. ear vein. Sits quietly when released; otherwise no perceptible effects in five minutes.

11: 36. 30 mg. procain × kg. in 10 parts N. S., ear vein; only feeble convulsive twitchings; then lies fully extended.

11: 38. First signs of recovery; raised head.

11: 49. Flat on belly; head nearly down to floor; able to raise it when stimulated.

11: 55. Dozing.

12: 02. Lies flat; respiration about 100 per minute.

12: 56. Lies flat; head to floor.

1: 55. Lies quiet, but able to get about.

3: 30. Hopping about floor; appears normal.

Local Anesthetics: Procain and morphine: Rabbits.

March 5, 1920:

6. Brown rabbit, 2.3 kg.

2: 45 p. m. 25 mg. procain × kg. into ear vein in 10 parts N. S., at once. Released; convulsion at once lasting about one minute; normal in about ten minutes (apparently. at least).

3: 13. 2 mg. morphin sulphate × kg. into ear vein in 50 parts N. S. Slight depression; little more than sufficient to cause animal to remain quiet.

3: 18. 25 mg. procain per kg. as previously. Feeble convulsion at once on being released.

3: 25. Lies on side fully extended; complete muscular relaxation.

3: 28. Lies flat on belly head to floor; muscular relaxation less than at 3: 25.

4: 10. Condition almost the same as at 3: 28. Gradual return to normal. Depression of respiration shown in shallow breathing.

Brown rabbit, 1.66 kg.

2: 12 p. m. 12 mg. morph. sulph. × kg. as in preceding.

2: 13. Beginning depression; head to floor after half minute.

2: 19. 25 mg. procain × kg. as in preceding experiment. Only slight convulsive tremors; respiration ceased.

2: 20. Few respiratory movements in a group. Artificial respiration.

2: 23. Heart beating feebly; gradually failing despite artificial respiration.

These experiments on rabbits with procain alone and with morphin, and with morphin and atropin, show that the morphin, and morphin and atropin, cause severe depression even with doses as small as 2 mg. morph. sulph., or this amount with 1 mg. atropin sulph. (all doses per kg.), and that with doses of 12 mg. morph. sulph. the respiratory depression is profound and lasting.

The convulsions following the administration of procain are *usually* less intense or less persistent in those rabbits which have previously had such large doses of morphin, but the depression is far more lasting and the fact that the convulsions are less is no indication for the simultaneous use of morphin; on the contrary, recovery is not so rapid and one rabbit died from a dose of 25 mg. of procain; a result that did not follow in any case of a normal animal so far as I recall, and certainly the death of this animal strongly suggests that the simultaneous use of morphin adds to the respiratory effect of procain. The heart continued to beat for several minutes after the respiration had ceased, but artificial respiration was without avail. The cause of death in man following small doses of procain may be independent of paralysis of the respiratory center, and in such cases it may be that the simultaneous use of morphin is not injurious.

COMMENT

The result of these investigations as to the use of morphin is rather negative. If anything it shows that the cat succumbs more readily to a poisonous dose of both drugs. In the human being there is in many instances a state of apprehension to operations, which if allayed by morphin might be of value. Hence we believe that its use is not contraindicated and should be further tried.

COMPARISON OF LOCAL AND GENERAL ANESTHESIA

Your committee finds that local anesthesia is the method of choice by practically all American rhinologists; there is less hemorrhage, far greater safety especially when operating near the cribriform plate, and the dangers of toxicity will compare favorably in the use of the local anesthetic in preference to any general anesthetic. Even the nitrous oxid and oxygen combination, looked on by many as absolutely safe, has a record of deaths that destroys that assuredness; one observer has resported thirty-seven deaths from this "safest" anesthetic.

In throat operations the unanimity is not so great, a small minority adhering to general anesthesia, for greater rapidity in work and ease and comfort to the operator.

The majority operate under local anesthesia and declare themselves to be entirely content therewith.

The ideal anesthetic having the effects of cocain and absolutely nontoxic has not been found, and we realize that the successful anesthetic of today may be discarded on the morrow; nor are we averse to the reporting of those which seem successful at the moment, but we deem it essential that once having so recorded and endorsed it, especially if unqualified, that the same publicity should be made in the event that one has ceased using the drug for grave and important reasons.

It should be understood that this entire report refers to *adults only*.

SUMMARY

As a result of the study of the literature, clinical experiences and animal experimentation, we conclude that:

1. There is a remarkable similarity in the clinical effects and animal experimentation.

2. None of the synthetic products equals cocain in its local effect when applied to the mucous membrane.

3. These synthetic products may be freely injected if slowly done, in proper doses in unlimited quantities.

4. Fatalities either occur immediately or not at all.

5. The drug is eliminated in the liver.

6. The greatest danger lies in too rapid injection or entering a vein.

7. A peculiar susceptibility which we term idiosyncrasy does exist, as the drug enters into the circulation so rapidly that death is almost immediate.

8. A further study of the toxicity of these local anesthetics will result in definitely establishing the causes of death.

9. Local anesthesia is undoubtedly the choice of methods by all American rhinologists in nose operations.

10. It is also the choice of a very large proportion of American laryngologists in throat operations.

11. There is a small number who believe that tonsil operations particularly are best performed under general anesthesia.

12. The dangers of hemorrhage during tonsil operations under local anesthesia are no greater than under general.

13. There is no greater danger from postoperative hemorrhage under local than under general anesthesia.

14. The previous administration of morphin requires further investigation.

SUGGESTIONS

Your committee suggests that all operations be performed with the patient recumbent, beginning with the first application of the local anesthetic, except in sinus operations, where the head and shoulders may be elevated to an angle of 45 degrees and the table raised so that the proper direction may be maintained.

Each operation to be preceded by a hypodermic injection of morphin and atropin and the patient kept in hospital.

In nose operations, application of adrenalin first, followed by cocain applied and the injection of the synthetic drug *introduced slowly.*

In throat operations applications of a 5 or 10 per cent. solution of cocain, followed by the *slow injection* of the synthetic product.

Where there may be a suspicion of possible danger, one-fourth of the amount of anesthetic to be used at the time of operation should be applied and the patient watched for possible toxic effects.

Among the cases included in these latter are those with evident cardiac disease, Basedow, or other disturbances of internal secretion.

CONCLUSIONS

Local anesthesia is the ideal method of operating for affections of the nose and throat.

None of the dangers that have been mentioned are any greater than those following general anesthesia.

The question of toxicity has heretofore not been sufficiently studied, and we are grateful to the Council on Pharmacy and Chemistry of the American Medical Association for proposing this investigation, and while the preparation of this report has entailed much labor, practically constant for nine months, your committee feels that it will not have been in vain if we have thereby added to our knowledge of the causes of fatalities, in thus preventing their occurrence in the future, to say nothing of the exceeding value that such formal conclusions will have to some colleague who may hereafter be confronted with one of these most distressing results.

RECOMMENDATIONS

In order that our work may be constructive, your committee recommends that a permanent committee on Toxicity Following Local Anesthesia be formed with the secretary of the section as a member, and that the secretary mail a copy of these resolutions to each registered member of the section, asking that reports of fatalities hereafter occurring should be promptly reported to him for further study by the committee.

This would require the cooperation of every member, and in return, the committee would pledge itself to present to any participating member the result of its findings in the form of a sworn affidavit for his support in the event of a fatality, thereby preventing possible legal action, at any rate, the assurance of cooperation and support from an independent source.

Your committee further recommends that a change be made from the old method of introducing new local anesthetics.

The method heretofore has been that clinical data were furnished by medical men chosen by the manufacturer, the Council on Pharmacy and Chemistry making its studies, and the product then, if favorably reported on, placed on the market. We would suggest that clinical investigation should be made by clinicians of our own choosing directly after the pharmacologist has made his investigation.

As such investigation requires much personal attention with careful annotation of the effects of the drug and a reasonably prompt report of the clinical investigation, we would suggest that a fund be placed in the hands of the Therapeutic Research Committee to properly reimburse the physicians who are making these studies, devoting many hours of their time thereto.

In presenting this report, your committee desires to state that its statements as to the advantages and disadvantages of local anesthesia are entirely impersonal; no one's individual preference is expressed, but rather a composite expression of the views now held by all rhinologists and laryngologists.

No one local anesthetic is favored, and a far and impartial statement is made of the merits or demerits of each.

In presenting suggestions for the methods of operating safely under local anesthesia, we have combined those endorsed by some of the most earnest and active of our colleagues in the profession.

From the great mass of evidence obtained from the writings of and many personal interviews with the clinician and the pharmacologist, we have carefully sifted the evidence and based our statements thereon.

Our recommendations for such improvement as may tend toward a clearer knowledge of the subject than now obtains, and the institution of newer methods in the introduction of new anesthetics, as against the older and more archaic methods are our own and express our verdict after careful study of the mass of evidence we have gathered.

Finally we recommend the use of the soluble tablets of cocain for making the solution freshly as required, one to be dissolved in hot sterile water by the operator. Each 240 minims of this solution makes a ½ per cent. solution. The unnecessary deaths quoted from "mistake of druggist," or "of nurse" would thus be avoided.

While the work of your committee has been very laborious during these many months, it has been made easier by the stimulus of the hearty cooperation, encouragement and approval of Prof. Torald Sollmann, chairman of the Therapeutic Research Committee, as also of Prof. Robert A. Hatcher, of the Department of Pharmacology of the Cornell Medical College, whose scientific zeal and great willingness to assist and perform new animal investigations have been a source of great inspiration.

Our sincere thanks are due to Dr. Cary Eggleston for his cooperation in assembling the résumé of preceding literature on animal experimentation; to Dr. T. F. Galloway for much assistance in abstracting such literature as was suggested; to the members of this section whose replies to the questionnaire have been of exceeding value and added much to our opportunities for study; and to the Council on Pharmacy and Chemistry of the American Medical Association, which voted a sum of money to defray the incidental and necessary expenses incurred in the preparation of this report.

Your chairman wishes also to extend his personal thanks to his associates on the committee, Dr. Skillern and Dr. Sonnenschein, for their aid in promptly assembling the investigations assigned to them and their most hearty cooperation.

If, as a result of our labor, we have added to our knowledge of this important subject, eventually resulting in the saving of human life, thus adding to the value of our services to humanity, your committee will feel itself amply repaid.

BACILLARY DYSENTERY IN CHILDREN *

WILBURT C. DAVISON
BALTIMORE

(From The Johns Hopkins Hospital Bulletin, July, 1920, p. 225)

INTRODUCTION

Bacillary dysentery is a disease chiefly affecting babes and soldiers.† In the former it sometimes masquerades under the names of summer diarrhea, infectious diarrhea, colitis or ileocolitis, which do not have the prestige and importance that is attached to the more ominous title "dysentery." That this disease in children is caused by *B. dysenteriae* has long been known,[1, 2, 3, 4, 5, 55] and is adequately confirmed by the bacteriological findings in our series of cases. The name "summer diarrhea" is particularly misleading. One might as well discuss "winter cough" as was done in 1872[6] and neglect to differentiate primary and secondary pneumonia from bronchitis. There are many types of intestinal disease that occur during the warm months and there is no reason for classing them all together, for dysentery stands out sharply on clinical as well as bacteriologic grounds.

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* From the Department of Pediatrics and the Department of Pathology of The Johns Hopkins University and The Harriet Lane Home of The Johns Hopkins Hospital, Baltimore, Md.

† The results of an investigation of an epidemic of bacillary dysentery in our troops in France (A. E. F.) will appear in a later article.

1. Duval, C. W., and Bassett, V. H.: The Etiology of the Summer Diarrheas of Infants: a Preliminary Report, Am. Med., Philadelphia, **4:** 417, 1902. Also: Stud. Rockefeller Inst. M. Research, N. Y., 1904, 1.

2. Flexner, S., and Holt, L. E., *eds.:* Bacteriological and Clinical Studies of the Diarrheal Diseases of Infancy, with Reference to the Bacillus dysenteriæ (Shiga), Stud. Rockefeller Inst. M. Research, N. Y., 1904, 1-202.

3. Tenbroeck, C., and Norbury, F. G.: The Presence of B. Dysenteriae, B. Proteus Vulgaris, B. Welchii and Morgan's Bacillus No. 1, in the Stools of Cases of Infectious Diarrhea, Boston M & S. J. **173:** 280-284, 1915. Idem: B. Dysenteriae as a Cause of Infectious Diarrhea in Infants, Boston M. & S. J. **174:** 785-788, 1916.

4. Slawik, E.: Serologische und klinische Beiträge zur Kenntnis der Dysenterie der Säuglinge, Jahrb. f. Kinderh., Berl., 1919, n. F. **90:** 119-140.

5. Ohno (quoted by K. Shiga): Bacillary Dysentery. *In:* Mod. Med. 2. ed. (Osler and McCrae), Philadelphia and New York **1:** 776, 1913. Hiss, P. H., and Russell, F. F.: A Study of a Bacillus Resembling the Bacillus of Shiga from a Case of Fatal Diarrhea, with Remarks on the Recognition of Dysentery, Typhoid and Allied Bacilli, Med. Rec. **63:** 357, 1903. Park, W. H.: On the Interpretation of Reactions of Agglutination Among the Bacilli of Dysentery, Med. Rec. **63:** 358-359, 1903. Zahorsky, J.: B. Dysenteriae in Infants, St. Louis Courier Med. **27:** 1902. Kendall, A. I.: Observations on the Etiology of Severe Summer Diarrheas of Bacterial Causation, Boston M. & S. J. **169:** 754-756, 1913. Martini, E., and Lentz, O.: Ueber die Differenzirung der Ruhrbacillen mittels der agglutination, Ztschr. f. Hyg. u. Infectionskrankh. **41:** 540-558, 1902. Shiga, K.: Ueber den Dysenteriebacillus (Bacillus Dysenteriae), Centralbl, f. Bakteriol., etc. **24:** 817-828, 1898. Vedder, E. B., and Duval, C. W.: The Etiology of Acute Dysentery in the United States, J. Exper. M. **6:** 181-205, 1902. Kruse, W.: Ueber die Ruhr als Volkskrankheit und ihren Erreger, Deutsch. med. Wchnschr. **26:** 637-639, 1900. Flexner, S.: On the Etiology of Tropical Dysentery, Philadelphia M. J. **6:** 414-424, 1900. Cuervo, A. M.: Bacillary Dysentery, Rev. med. d. Uruguay **22:** 117, 1919. Hastings, Pease, Shaw and Jehle: *In:* M. Pfaundler and A. Schlossmann: The Diseases of Children, Eng. transl. ed. by H. L. K. Shaw and L. La Fétra, Philadelphia and London, J. B. Lippincott Co. **3:** 298, 1908. Wollstein, Martha: The Dysentery Bacillus in a Series of Cases of Infantile Diarrhea, J. Med. Research **10:** 11-20, 1903-1904. Leiner, K.: Ueber bazilläre Dysenterie, speziell im Kindesalter, Wien. med. Wchnschr. **17:** 738-745, 1904.

SOURCE OF THE MATERIAL

The material for this report consisted of the study of 134 cases of diarrhea. Eighty of these were in the wards of this hospital from March, 1919, to February, 1920, nine cases in the Thomas Wilson Sanitarium, Mt. Wilson, Maryland, during June and July, 1919, and 45 cases in the private practices of several physicians in Birmingham, Alabama, from July 14 to August 9, 1919.*

CLINICAL DATA

Among the total 134 cases of diarrhea, 71 were diagnosed by the clinicians as cases of dysentery (ileocolitis).

Clinically, the disease is a definite type, occurring usually in well-nourished and previously healthy children whose ages ranged from 3 months to 11 years (see Table 1). The ages of the Birmingham patients were higher than those of Baltimore. This may pos-

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55. Torrey, J. C.: A Comparative Study of Dysentery and Dysentery-Like Organisms. Pt. 1, Bacilli Producing a Typical Reaction in Litmus Milk; Pt. 2, Dysentery-Like Organisms which Produce an Atypical Reaction in Litmus Milk and Should Probably be Included within the Group of Pseudodysentery Bacilli, J. Exper. Med. **7**: 365-385, 1905.

* These latter cases were studied by Dr. Harold L. Higgins and myself at the request of Dr. John Howland and with a grant of money from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association. Through the courtesy of Dr. J. D. Dowling, Health Officer of Birmingham, Alabama, and Dr. J. R. Bean, we were given all the facilities in the Board of Health Laboratory. Through the efforts of Drs. Thomas D. Parke, Russell Callen, Courtney W. Shropshire and fifteen other physicians, we were able to obtain stool specimens from several cases of diarrhea in infants in Birmingham and its environs. Our sincere thanks are due these physicians and also Drs. Catherine Creighton, Harold L. Higgins, L. V. Rosenthal and C. E. Wagner for assistance with the bacteriological work.

[6] Dobell, H.: On Winter Cough Catarrh, Bronchitis, Emphysema, Asthma: a Course of Lectures Delivered at the Royal Hospital for Diseases of the Chest, London, 1872, J. & A. Churchill, 238 pp. 8°.

sibly be explained by the fact that in the former breast feeding was a more general rule and therefore the younger children escaped infection.

With one or two doubtful exceptions, none of the patients was exclusively breast fed, but received supplementary feedings of whole milk, proprietary foods or general diet.

TABLE 1.—AGES OF BALTIMORE AND BIRMINGHAM DYSENTERY CASES

	Ages of All Baltimore Dysentery Cases	Ages of All Birmingham Cases	Ages of Fatal Baltimore Cases	Ages of Fatal Birmingham Cases
Average........	16 months	25½ months	11 months	15 months
Mean (excluding wide extremes)	14 months	17 months	5½ months	12 months
Minimum.......	3 months	6 months	4 months	6 months
Maximum......	36 months	132 months (11 years)	36 months	30 months

TABLE 2.—CHARACTER OF ONSET OF BALTIMORE DYSENTERY CASES *

	Sudden Onset with Fever	Insidious Onset	Dysenteric Infection Occurring During Course of Other Disease	Vomiting at Onset	Irritability and Nervous Symptoms at Onset	Convulsions at Onset	Loss of Appetite and Refusal of Food at Onset	Previous Intestinal Disease (Several Months Prior to Dysenteric Infection)	Average Time of Appearance of Blood in Stool After Onset
No. of cases	23	4	2 cases †	14	14	6	10	3	2.3 days
Percentage	79	14	7	52	48	21	34	10	

* Analysis based on 29 cases in which the histories were complete.
† 1 osteomyelitis, 1 scurvy.

The onset was usually characteristic and sudden (see Table 2). The child lost his appetite and showed evidence of having fever. Irritability, vomiting and convulsions were frequent initial symptoms. Within a few hours there was an increase in the number of stools. These were at first fecal, rarely green, becoming blood-tinged on the second day, and within four days consisted almost entirely of blood and mucus. The number of stools ranged from three to thirty a day. The size of the stool was usually small and seemingly out of proportion to the patient's straining efforts.

The fever subsided in most cases by the fifth day. The patients were usually quite all, crying feebly when disturbed, were drowsy and apathetic, after their initial irritability had ceased, and had little or no appetite. Loss of sleep due to the frequent and often painful bowel movements was a distressing factor. Dehydration and marked loss of weight was evident by the end of the first week.

In the patients that recovered, the blood disappeared from the stools during the second week and the diarrhea abated by the fourteenth day.

Such was the course in 79 per cent, of our cases. In 14 per cent. (4 cases), however, the picture was misleading. With these four children, there was no sudden onset. They had been under observation for over one month with symptoms of intestinal indigestion; i. e., occasional vomiting and from three to six loose green stools a day which frequently contained mucus. Their weight was stationary or showed slight losses and they were malnourished. After several weeks of these symptoms, the stools became blood-tinged and were increased in frequency to nine or eleven a day. The temperature was slightly increased. This picture remained unchanged for several days and then the condition returned to the former state of mild diarrhea. During the period of bloody diarrhea *B. dysenteriae* (Flexner) was isolated from the stools of three of these four cases and agglutinins for *B. shigae* were demonstrated in the serum of the fourth Were these four cases instances of chronic dysentery that had become worse or did the bloody diarrhea represent a dysenteric infection that had become superimposed on a previously inflamed intestinal mucosa? The latter appears to be the more probable explanation.[7]

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[7] Zondek, S. G.: Ueber kombiniertes Auftreten von Infektionskrankheiten; 1, Typhus und Ruhr; 2, Fleckfieber und Rückfallfieber, Berl. klin. Wchnschr. **40**: 945-948, 1919.

Such cases must be distinguished from cases of intestinal indigestion in which the stools contain streaks of blood (usually bright blood) for only one or two movements and never longer than one day. This blood is probably the result of mechanical irritation of the lower rectum due to straining. The cultural results in such cases were negative.

In two other patients there were histories of intestinal indigestion several months previously, from which they had completely recovered. There were no agglutinins for *B. dysenteriae* in the serum of the only one that was tested during this earlier period; yet when he came into the hospital four months later with clinical dysentery, the reaction was positive, so that it would seem probable that the previous diarrhea had no connection with the later dysenteric infection.

However, it must not be forgotten that in adults a mild nonbloody diarrhea lasting only 24 hours has been proven to be due to dysenteric infection.[8] There is no reason to suppose that similar cases cannot arise in children.

In two other cases (7 per cent.) the diagnosis of dysentery was marked by the presence of other diseases; in one by scurvy (poven by roentgen ray) and the other by osteomyelitis. *B. dysenteriae* (Flexner) was isolated from the stools of each of these cases.

In the dysentery cases in Baltimore and Birmingham there were 67 white children with 14 deaths and 4 colored with 1 death, a total mortality of 21 per cent.*

An analysis of the ages of the fatal cases in Table 1 would seem to indicate that the younger patients were more vulnerable.

The leukocyte count of the Baltimore cases was slightly increased.[9] The mean was 13,000 for all cases and 13,700 for those that died. Wide variations, without obvious cause, such as otitis media, were, however, to be noted. In fact the leukocyte count was of little or no diagnostic importance.[10]

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* The mortality for these 1919 cases was lower than that of former years, for among 114 cases treated at the Harriet Lane Home from 1912 to 1918 inclusive, there were 33 deaths, a mortality of 29 per cent.

[8] Graham, D.: Some Points in the Diagnosis and Treatment of Dysentery Occurring in the British Salonika Force, Lancet **1**: 51-55, 1918.

[9] Knox, J. H. M., Jr.: A Contribution to the Study of Summer Diarrheas of Infancy, J. A. M. A. **41**: 173-179, 1903.

The physical findings were of little assistance. In only one case was the spleen palpable. The liver was occasionally somewhat enlarged and tender. Abdominal tenderness was not marked. Five of the patients were emaciated on admission and two had obvious rickets.

Complications were infrequent.[11] Pyelitis occurred in one case, *B. coli communis* being isolated from the catheterized urine. Otitis media developed in two cases. Hyperpnea [12] occurred in three cases.

It is frequently stated that after recovery from dysentery (ileocolitis) the feeding of the small patients may become a difficult problem. This has occurred in very few of our cases. Occasionally there was persistent refusal to take food so that gavage was necessary for a few days. With these exceptions, all of the other patients took their feedings well and showed a steady increase in weight; after three months they were well-nourished, exceedingly healthy children. No case became chronic.

Clinically (as well as bacteriologically) there was no difference between the cases we saw in Birmingham and those that we have studied in Baltimore. However, the ratio of dysentery (ileocolitis) cases to those of simple noninfectious diarrhea was 8 to 1 in Birmingham and 1 to 2 in Baltimore.

The postmortem diagnoses in practically all of the children that died of uncomplicated dysentery were definite diphtheritic or ulcerative or acute necrotizing enterocolitis depending on the nomenclature of each pathologist. In fatal cases in children suffering from malnutrition, scurvy, rickets or other diseases in which dysentery had acted as the last straw, as a complicating and not the primary cause of death, the postmortem diagnoses of the intestines ranged from normal to hyperemia or slight ulceration of the lymph follicles of the lower third of the ileum and the colon. In other words, the necropsy revealed the severity of the infection and the virulence of the causative organism. It is frequently stated that the degree of ulceration of the intestine depends on the length of the illness. This statement did not appear to be true in this series of cases.

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[10] Ryder, C. T.; Richards, E. T. F.; Peabody, A. H.; Canavan, M. M.; Southard, E. E., and McGaffin, G. G.: Report of an Epidemic of Bacillary Dysentery at the Danvers State Hospital, Massachusetts, in 1908, Boston M. & S. J. **161**: 679-714.

[11] Womack, N. C.: Ileocolitis (Proc. South. Med. Assn., Nov. 10-13, 1919), J. A. M. A. **73**: 1797, 1919.

[12] Howland, J., and Marriott, W. McK.: Acidosis, Penn. M. J. **21**: 429, 1917-1918.

The necropsy of one case of Flexner dysentery of but three days' duration showed the ulcers and membrane formation of typical acute enterocolitis.

Three days after the death of this patient, his brother developed clinical dysentery which cleared up after an illness of two weeks. *B. dysenteriae* (Flexner) was isolated in this case also.

Similar findings occurred in the remaining uncomplicated fatal dysentery cases. Occasionally in addition to the ulceration of the lower third of the ileum and the colon, there were evidences of fatty degeneration of the liver. These probably correspond to the findings reported by Parke in cases in Birmingham.[13]

ETIOLOGY—BACTERIOLOGICAL AND SEROLOGICAL DATA DYSENTERY CASES

In Birmingham, Alabama, from July 14 to August 9, 1919, I cultured one or more specimens of the stools of forty patients with clinical dysentery. Only half of these were in the acute stage of the disease and from only thirteen did we obtain satisfactory specimens because all of the patients except two were in private homes scattered throughout the city and it was often impossible for us to be notified until the stool specimen had become too old to give reliable bacteriologic results.

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[13] Parke, T. D.: Acidosis: Retrospect and Discussion, South. M J. **11**: 721-727, 1918.

Of the thirteen cases from which we received satisfactory stool specimens, I recovered *B. dysenteriae* (Flexner)[*] in six cases, *B. dysenteriae* (Shiga) in one and *B. dysenteriae* (Kruse E)[14, 15] (or B. *dispar*)[16] in one. (A description of the technic employed is appended.) Positive agglutinins for *B. dysenteriae* (Shiga) were demonstrated in the sera of two additional patients. (The latter were brother and sister in a family in which one child had died the week previously of clinical dysentery and both parents were convalescent from dysentery.) In other words, in these thirteen cases, infection with *B. dysenteriae* was demonstrated in ten.

Of the thirty-one cases of clinical dysentery in the wards of this hospital, *B. dysenteriae* (Flexner) was recovered in eighteen. Positive agglutinins for *B. dysenteriae* (Flexner) were demonstrated in seven additional cases and for *B. dysenteriae* (Shiga) in one further case. Three were negative culturally but agglutination reactions were not done and two were negative bacteriologically and serologically. In other words, infection with *B. dysenteriae* was shown in over 83 per cent. of the cases of clinical dysentery in the Harriet Lane Home during the past year.

The ratio of Flexner to Shiga infections was 8 to 1. The Flexner bacillus is usually the more common type in children,[2, 53, 54, 55] In fact, some investigators[53, 56] found mixed infections with both varieties more frequently than the Shiga type alone.

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[*] By Dysenteriae (Flexner) I refer to the whole group of mannite fermenting dysentery bacilli.

14. Kruse: Neue Untersuchungen über die Ruhr, Deutsch. med. Wchnschr. **33:** 292-338, 1907.

15. Murray, E. G. D.: An Attempt at Classification of B. Dysenteriae, Based upon an Examination of the Agglutinating Properties of Fifty-Three Strains, J. Roy. Army Med. Corps **31:** 257-353, 1918.

16. Andrewes, F. W.: Dysentery Bacilli; the Differentiation of the True Dysentery Bacilli from Allied Species, Lancet, **1:** 560-563, 1918.

53. Knox, J. H. M., and Schorer, E. H.: The Relation of Types of Diarrhea in Children to Strains of B. Dysenteriae, Jour. Exper. Med. **8:** 377, 1906.

54. Smillie, G. W.: Epidemiology of Bacillary Dysentery, and Discussion by H. I. Bowditch, New York M. J. (etc.) **104:** 1073-1074, 1916.

tors[53, 56] found mixed infections with both varieties

56. Gay, F. P., and Duval, C. W.: Acute Dysentery Associated with Two Types of B. Dysenteriae, Univ. of Penn. Med. Bull., Philadelphia **16:** 177, 1903-4.

The dysentery agglutination reaction assists the diagnosis of dysentery.[3, 4] It is essential, however, for each worker to standardize his cultures and technic with normal control sera, especially in Flexner infections, inasmuch as the agglutinability of different strains of this organism varies considerably. Freshly isolated strains should not be used, for their agglutinability is inconstant. I have used the standard agglutinable cultures issued by the Department of Pathology, University of Oxford, on behalf of the Medical Research Committee (England) and also formalized cultures made according to Prof. Georges Dreyer's[17] instructions and found (Table 3), as he states, that macroscopic dysentery agglutination by a patient's serum with standard agglutinable cultures or comparable preparations in a dilution 1:30 (6-8 units) is suggestive and a titer of 1:50 (10 or more units) is diagnostic of previous or present dysentery infection.* In all patients from whom an agglutinable *B. dysenteriae* was recovered and whose serums were tested, specific agglutinins were found. In only three patients who were not, at that time, suffering from bloody diarrhea did I demonstrate dysentery agglutinins (*vide infra*).

The literature on this subject is contradictory. It is frequently stated that the patient's serum reactions in dysentery are worthless.[18] It is just as frequently claimed that they are infallible. Glynn and his co-workers[19] give an excellent analysis of the various methods and insist that agglutination, if done with standardized methods, is reliable.

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* In infants and young children a positive dysentery agglutination even at a dilution of 1:20 early in the disease is very suggestive. In my experience nonspecific agglutination in young children is infrequent.

[17] Davison, W. C.: The Diagnosis of Enteric Fever (Typhoid and Paratyphoid A and B) by Agglutination Tests, J. A. M. A. **66**: 1297-1300, 1916. Idem: Report on Prophylactic Vaccination with B. Typhosus, B. Paratyphosus A, and B. Paratyphosus B, J. Lab. & Clin. M. **2**: 607-617, 1916-17.

[18] Shiga, K.: Bacillary Dysentery. *In:* Mod. Med. 2 Ed. (Osler and McCrae), Philadelphia and New York, **1**: 778, 1913. Fildes, P.: Report upon Recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915-1916. Med. Research Comm. Special Rep., Ser. No. 6, London, 1917.

[19] Glynn, E.; Berridge, E. M.; Foley, V.; Price, M., and Robinson, A. L.: A Report upon 2,360 Enteritis "Convalescents" Received at Liverpool from Various Expeditionary Forces, Med. Research Comm. Special Rep., Ser. No. 7, London, 1918.

TABLE 3

		Agglutination Reactions of the Patient's Serum			Type of Dysentery Bacillus Isolated from Stool Culture
Case No.	Age at Onset	Days After Onset	Flexner Culture	Shiga Culture	
23	7 mos.	2	? 1/25	0 1/25	
		4	± 1/25	0 1/25	
		9	+ 1/50	0 1/20	
82	5 mos.	6	± 1/200	+ 1/50	
58	4 mos.	6	0 1/20	0 1/20	Flexner (inagglutinable)
11	24 mos.	7	+ 1/500	0 1/29	Flexner
10	15 mos.	9	+ 1/200	Not done	Flexner
16	36 mos.	9	+ 1/100	0 1/20	
		15	+ 1/250	+ 1/50	
78	11 mos.	13	+ 1/20	Not done	Flexner
54	30 mos.	14	+ 1/100	0 1/20	Flexner
79	9½ mos.	17	+ 1/200	0 1/20	Flexner
		21	+ 1/100	0 1/20	
80	26½ mos.	19	+ 1/200	0 1/20	Flexner
38	....	..	+ 1/200	0 1/20	Flexner (urine culture)
46	36 mos.	21	+ 1/1000	0 1/20	Flexner
[illegible]	19 mos.	134 before onset	0 1/25	0 1/25	
		21 after onset	+ 1/50	0 1/20	
		158 after onset	+ 1/20	0 1/20	
77	8 mos.	26	± 1/50	0 1/20	Flexner
		76	+ 1/50	0 1/20	
42	22 mos.	27	+ 1/100	0 1/20	
44	23 mos	37	± 1/20	0 1/20	
50	3½ mos.	38	0 1/20	0 1/20	
		140	0 1/20	0 1/20	
59	14½ mos.	39	+ 1/100	0 1/20	
		138	± 1/50	0 1/20	
63	6½ mos.	68	+ 1/20	0 1/20	Flexner
		172	0 1/20	0 1/20	
56	3 mos.	90	+ 1/20	0 1/20	Flexner
45	17 mos.	176	0 1/20	0 1/20	Flexner
41	14 mos.	184	± 1/20	0 1/20	Flexner
39	23½ mos.	200	± 1/50	0 1/20	Flexner
40	11 mos.	205	± 1/200	0 1/20	Flexner
2	48 mos. (4 years)	18	0 1/25	± 1/50	
4	132 mos. (11 years)	20	0 1/25	+ 1/125	
37	14½ mos.	45	0 1/20	+ 1/50	

Five Flexner antigens (cultures) English (Murray) types V, W, X, Y and Z were used for agglutination, and the reactions denote the highest titers for any one of them. All of these patients had clinical dysentery.

The confusion on this question is readily explained. If a freshly isolated living culture of *B. dysenteriæ* is used for agglutination, a positive result may be obtained with a known serum in a dilution of 1 : 500. A few weeks later the same serum and a subculture of the same *B. dysenteriae* strain may give a positive agglutination at an end titer of only 1 : 100. In other instances a freshly isolated *B. dysenteriae* culture may be agglutinated only at the end titer of 1 : 100. Yet after a few weeks of laboratory cultivation, positive agglutination may be noted with the same serum at a titer of 1 : 500.

Obviously, then, if a laboratory worker tests the serums of normal persons with either of these two types of culture, his figures are useless for later comparison with the agglutination results of patients with dysentery.

If living cultures of older laboratory strains are used, this difficulty may be remedied though not cured, for although strains that have been artificially cultivated for long periods have reached quite a constant level of agglutination, yet there is still some variation. Furthermore, the emulsions of these living cultures will vary in opacity and thickness from day to day. This will also give confusing results for agglutinations with the same serum, and emulsions of the same strain which are of different opacity will give different end titers. Thinner emulsions, within certain limits, will be agglutinated by the same serum in higher dilutions than those that are more opaque.[20]

These are some of the arguments Dreyer, Ainley, Walker, Gibson and others [21, 17, 22] have advanced in support of the Oxford Standard agglutinable cultures. It is not to be denied that conflicting results have been obtained by some investigators[23] with Dreyer's method. In the main, however, they have been most satisfactory and results in different laboratories are thus comparable.

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[20] Krumwiede, C.: Personal communication.

[21] Dreyer, G.: Om Anvendelse af draebt kultur til Widal-Reakton (The Employment of Dead Cultures for the Widal Reaction), Hosp.-Tid., Kobenh., 4, R. **14:** 532-542, 1906. Idem: Discussion on Immunity: Agglutination, Proc. Brit. Med. Assn., Section of Pathology, Brit. M. J. **2:** 564-567, 1904. Idem: Widal's Reaction with Sterilized Cultures, J. Path. & Bacteriol. **13:** 331-337, 1909. Idem: Discussion on Paratyphoid Fever, Proc. Roy. Soc. Med. **9:** Sec. Med., 9-15, 1915-16. Dreyer, G., and Jex-Blake, A. J.: On the Agglutination of Bacteria, J. Path. & Bact. **11:** 1-28, 1906. Dreyer, G., and Walker, E. W. A.: Observations on the Production of Immune Substances, J. Path. & Bact. **14:** 28-38, 1909. Idem: The Diagnosis of the Enteric Fevers in Inoculated Individuals by the Agglutinin Reaction, Lancet **2:** 419-423, 1916. Dreyer, G.; Walker, E. W. A., and Gibson, A. G.: Typhoid and Paratyphoid Infection in Relation to Antityphoid Inoculation, Together with Remarks on the Importance of the Use of Prophylactic Paratyphoid Inoculation, Lancet **1:** 324-328, 1915. Dreyer, G.; Gibson, A. G., and Walker, E. W. A.: Further Remarks on Agglutination Tests in Inoculated Persons, and the Influence of Febrile Conditions on Inoculation Agglutinins, Lancet **1:** 766-768, 1916. Dreyer, G., and Torrens, J. A.: Paratyphoid (correspondence), Lancet **2:** 1369-1370, 1915. Dreyer, G., and Inman, A. C.: The Agglutination Curve and Its Importance in the Diagnosis of Typhoid and Paratyphoid Fevers in Inoculated Persons, Lancet **1:** 365-369, 1917.

[22] Davison, W. C.: The Superiority of Inoculations with Mixed Triple Vaccine (B. Typhosus, B. Paratyphosus A, and B. Paratyphosus B), Arch. Int. Med. **21:** 437-509, 1918.

Furthermore, it is not as clearly recognized as it should be that while there is but one type of Shiga dysentery bacillus, there are at least five subdivisions of the mannite-fermenting group. Murray[15] has designated these as *B. dysenteriae* (Flexner) types V, W, X, Y and Z (based on agglutination) and has dropped the older subgroup names of Flexner, Harris, Hiss-Russel-Y and Strong (based on the differences in fermentation of maltose and saccharose). If agglutination reactions with patients' serums are done with only one or two types of the Flexner bacillus as antigens, obviously some positive tests may be missed.[24]

I have studied eighty-nine cultures * of mannite-fermenting dysentery bacilli isolated from these cases and was able to differentiate them culturally into seven divisions on the basis of the fermentation of maltose, saccharose, dulcite and rhamnose. These cultures were also differentiated serologically by their agglutination reactions with Murray's five (English) Flexner monovalent rabbit serums, types V, W, X, Y and Z. These serologic divisions did not run parallel with the fermentative divisions. A general correspondence existed between the serologic types of the organisms isolated from the patient's stool and agglutination reactions of his serum. One or the other and not both of these methods of division must be adopted. The classification by agglutination is advisable, because it is simpler and more rapid. The results of the agglutination reactions of the patient's serum may be expressed in the same terms as the serologic typing of the organisms from his stool. Fermentation is less constant and gives rise to more divisions than there are carbohydrates.

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* A paper on the "Divisions of the So-Called Flexner Group of Dysentery Bacilli" will appear later.

[23] Leishman, W.: Discussion on Paratyphoid Fever, Proc. Roy. Soc. Med., London **9**: Med. Sect., 17-21, 1915-1916. Strong, R. P., *ed.:* Trench Fever, Report of Commission, Medical Research Committee, American Red Cross, Oxford, 1918, Oxford Univ. Press, 446 pp. 4°.

[24] Dudgeon, L. S., *ed.:* Studies of Bacillary Dysentery Occurring in the British Forces in Macedonia, Med. Research Comm. Special Rep., Ser. No. 40, London, 1919.

The serologic reactions of these type serums, as Murray states, show cross-agglutination to a greater or less extent but they indicate that there are five antigens V, W, X, Y, Z and probably others, one or more of which predominates in a given strain. Polyvalent diagnostic and therapeutic dysentery serum are practically worthless unless they include antibodies for the more common of these types.

In the agglutination reactions recorded in this paper, eight dysentery antigens were used, i. e., Shiga, Flexner types V, W, X, Y and Z, *B. dispar* (or Kruse E) (a lactose fermenting dysentery) and *B. ambiguus* Schmidtz[25] (an indol producer which forms acid and no gas in dextrose and rhamnose).

As will be seen in Table 3, suggestive agglutination was noted in one case (No. 23) on the second day,[10] the titer rising on the fourth and ninth days. The maximum is probably on the seventeenth to twenty-first day as it is in typhoid. From the stool of one patient (No. 58) a dysentery (Flexner) bacillus, that did not agglutinate with any of the five diagnostic type serums, was isolated and this patient's blood serum had no agglutinins for the V, W, X, Y and Z stock cultures, suggesting that there are additional types in the Flexner group.[24] Agglutinins for both Shiga and Flexner groups were noted in only two cases.[24, 26, 27]

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[25] Schmitz, K. E. F.: Ueber einen bisher noch nicht bekannten Krankheitserreger aus der Dysenteriegruppe, Münch. med. Wchnschr. **65:** 1571, 1918. Idem: Ein neuer Typus aus der Gruppe der Ruhrbazillen als Erreger einer grösseren Epidemie, Ztschr. f. Hyg. u. Infectionskrankh. **84:** 449-516, 1917.

[26] Lancelin, R., and Rideau, I.: Recherches épidémiologiques, bactériologiques et anatomo-pathologiques au cours d'une épidémie de dysentérie bacillaire observée à Brest en 1916, Arch. de méd. et pharm. nav., Par. **104:** 360, 1917; **105:** 47, 1918. Speares, J., and Debono, P. P.: Agglutination in Bacillary Dysentery, J. Roy. Army Med. Corps, London **32:** 430-440, 1919.

[27] Rajchman, L., and Western, G. T.: Report on 878 Cases of Bacillary Enteritis, Med. Research Comm. Special Rep., Ser., No. 5, Lond., 1917.

Agglutinins persisted in several cases longer than four months and in three cases tested after six months they were still present.[24]

CONTROL SERIES (63 CASES OF SIMPLE DIARRHEA * AND 100 NORMAL CHILDREN)

In addition to the 71 cases of clinical dysentery just described there were 63 patients studied who developed increased frequency of stools at one time or another during the course of other illnesses. The clinical diagnoses of these latter were gastro-intestinal indigestion (47), typhoid fever (6), pyelitis (3), tuberculosis (2), hydrocephalus (1), eczema (1), bronchopneumonia (1), scurvy (1) and endocarditis (1).

The onset of the increased frequency of stools in each of these cases was insidious. There was no irritability or increased temperature. Several of the patients vomited occasionally, but loss of appetite was not obvious. Practically all gave histories of diet indiscretions especially of high sugar feeding. The stools were always fecal, usually green and contained no blood. Only in cases of long duration was mucus present in excess. In fact, in none of this group with the exception of the typhoid cases was there evidence of an acute intestinal infection.

The study of the stools of these 63 cases of simple * or noninfectious diarrhea, as well as those of 100 normal children, serves as an admirable control series for the studies on the cases of dysentery, for in none of the former (63 cases of diarrhea and 100 normal children) did we obtain *B. dysenteriae* from the stools. The serum of three patients among sixteen with diarrhea tested showed positive dysentery agglutinations. Two of these gave histories of bloody diarrhea some months before admission and, as shown in Table 3, agglutinins may persist in the blood six months after a dysenteric infection. The third had typhoid fever and there is evidence to show that one infectious disease may raise the nonspecific agglutinin titer.[27]

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* The term simple or noninfectious diarrhea or merely diarrhea is used in contradistinction to the bloody diarrhea of dysentery.

The negative cultural results in these cases of simple diarrhea and in normal children when contrasted with the positive findings in 83 per cent. of the cases of bloody diarrhea indicate plainly that children presenting a syndrome as outlined in Table 2 are suffering from bacillary dysentery.

Indeed it would seem that a correct differentiation of dysentery from diarrhea can be made in most instances on clinical data alone.

The bacteriologic and serologic results indicate that the Birmingham and Baltimore cases were of a similar type.

The results in Baltimore and Birmingham bear out the conclusions of Tenbroeck and Norbury in Boston in 1914 and 1915[3] that ileocolitis in children is due to infection with dytentery bacilli. Duval and Bassett[1] in 1902 and the Rockefeller Commission in 1903[2] demonstrated *B. dysenteriae* in a high percentage of cases that they called "summer diarrhea," but a clinical analysis of their cases shows that it was usually from the bloody and mucous stools of patients with typical dysentery that these organisms were isolated.

In fact, it would seem that ileocolitis and bacillary dysentery in children in Boston, Baltimore, Birmingham, New York and probably other cities are one and the same disease.

Some investigators have given significance to the presence of *B. welchii* (gas bacillus) in the stools of patients with diarrhea.[28] They have also gone fur-

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28. Kendall, A. I., and Smith, R. M.: A Rapid Presumptive Test for Diarrhea Caused by the Gas Bacillus, Boston M. & S. J. **163:** 578-579, 1910. Idem: Diarrhea in Infants Associated with the Gas Bacillus in the Stools, Boston M. & S. J. **164:** 306-308, 1911. Morse, J. L.: Infcetious Diarrhea, Am. J. M. Sc., Phila. **149:** 17-30, 1915. Sylvester, P. H., and Hibben, F. H.: The Relation of the Gas Bacillus to Infectious Diarrhea and Other Digestive Disturbances in Childhood, Arch. Pediat., N. Y. **32:** 457-472, 1915. Kendall, A. I.: The Relation of Bacteria to the Etiology of Summer Diarrhea in Young Children, Boston M. & S. J. **172:** 851-857, 1915. Hill, L. W.: The Etiology and Treatment of the Diarrheal Diseases of Infancy, J. A. M. A., Chicago **72:** 1653-1658, 1919.

ther and outlined special treatment and diets for this so-called "gas bacillus diarrhea." However, this was soon contradicted, but although Simonds,[29] Knox and Ford[30] and others[3, 31] have conclusively proven that *B. welchii* is an inhabitant of the intestines of normal children, some pediatrists are still influenced by its presence. I have often found *B. welchii* in normal stools and am convinced that it plays no part in the etiology of clinical dysentery or noninfectious diarrhea. Those who have studied war wounds realize that *B. welchii* does not produce dire results except in tissues where its supply of oxygen is limited. How can it be pathogenic in intestinal contents which are freely moving and well supplied with oxygen?

Perhaps the clearest analogy is found in tetanus. *B. tetani* is pathogenic in deep wounds yet it is common knowledge that this organism is frequently found in horse feces. Has any one accused the horse of "tetanus diarrhea"?

B. Morgan No. 1 has been asserted to be the cause of diarrhea in children, especially in England.[32, 33] In

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29. Simmonds, J. P.: Classification of the Bacillus welchii Group of Bacteria, J. Infect. Dis., Chicago **16:** 31-34, 1915. Stud. Rockefeller Inst. M. Research, N. Y. **2,** p. 10, 1904.

30. Knox, J. H. M., and Ford, W. W.: Note on the Occurrence of Bacterium welchii (Bacillus aërogenes capsulatus) in the Dejecta of Children, Johns Hopkins Hosp. Bull., Baltimore **26:** 27-28, 1915.

31. Sisson, W. R.: A Critical Review of Intestinal Bacteriology in Relation to Certain Diarrheas of Infants, Boston M. & S. J. **178:** 492-498, 1918.

32. Morgan, H. de R.: On the Bacteriology of the Summer Diarrhea of Infants, Brit. M. J., Lond. **1:** 908-912, 1906. Idem.: On the Bacteriology of the Summer Diarrhea of Infants (Second Communication), Brit. M. J., Lond. **2:** 16-19, 1907. Morgan, H. de R., and Ledingham, J. C. G.: The Bacteriology of Summer Diarrhea, Proc. Roy. Soc. Med., Lond. **2,** Epidemiol. Sec., 133-147, 1908-1909.

33. Lewis, C. J.: Bacteriology of Normal and Diarrheal Stools of Children in Birmingham, England, 1910, Loc. Gov. Board Reports, Med. Suppl., Appendix B, No. 2, 314, 1910-1911. Idem.: Bacteriology of Normal and Diarrheal Stools of Children in Birmingham, England, 1911, Loc. Gov. Board Reports, Med. Suppl., Appendix B, No. 2, 265, 1911-1912. Orr, T.: Bacteriology of Normal and Diarrheal Stools of Children in Shrewsbury, England, 1910, Loc. Gov. Board Reports, Med. Suppl., Appendix B, No. 5, 374, 1910-1911. Ross, S. M.: Bacteriology of Normal and Diarrheal Stools of Children in Manchester, England, 1910-1911, Loc. Gov. Board Reports, Med. Suppl., Appendix B, No. 3, 347, 1910-1911. O'Brien, R. A.: Bacteriology of Normal and Diarrheal Stools of Children in London, England, 1910, Loc. Gov. Board Reports, Med. Suppl., Appendix B, No. 3, 367, 1910-1911. Alexander, D. M.: Bacteriology of Normal and Diarrheal Stools of Children in Liverpool, England, 1911, Local Gov. Board Reports, Med. Suppl., Appendix B, No. 3, 288, 1911-1912. Idem: Bacteriology of Normal and Diarrheal Stools of Children in Liverpool, England, 1912, Local Gov. Board Reports, Med. Suppl., Appendix B, No. 4, 384, 1912-1913.

this series of cases I have found this organism frequently associated in the same stools with *B. dysenteriae.* I have found it in many cases of simple diarrhea in children and also in many adults. In no case, in which serological tests were made, did the patient's serum agglutinate *B. Morgan No. 1.* Tenbroeck and Norbury[3] and Thjotta[34] report similar results. As a matter of fact the British investigators found Morgan bacilli in the stools of many normal children. In very few of their diarrhea cases were the stools bloody and it is open to doubt whether they were dealing with cases similar to our clinical dysentery. Lewis[35] and Kligler[36] have shown that *B. Morgan No. 1* probably represents a wide group of organisms rather than a single type. I have carried out agglutination tests with four of Kligler's diagnostic rabbit serums and fifty strains of *B. Morgan No. 1* isolated from diarrheal and normal stools and found that there is no uniformity in results and that many strains are inagglutinable, while others are agglutinated by more than one serum.

It is generally agreed that in specific intestinal infections such as typhoid, paratyphoid and bacillary dysentery the causative organisms produce agglutinins in the patient's serum. Furthermore, *B. typhosus, B. paratyphosus and B. dysenteriae* are not found in the stools of normal cases except in occasional carriers who practically always give a history of having had typhoid or dysentery. *B. Morgan No. 1* does not fulfill either of these postulates so that it would seem improbable that this organism could be the specific cause of an intestinal infection like dysentery.

34: Thjotta, T.: On the Bacillus of Morgan No. 1.—A Meta-Colon Bacillus, J. Bact., Balt. **5:** 67-77, 1920.

35. Lewis, C. J.: Agglutination Reactions of B. Morgan Nos. 1 and 2 from Normal and Diarrheal Cases, Loc. Gov. Board Reports, Med. Suppl., Appendix B, No. 3, 375, 1912-1913.

36. Kligler, I. J.: The Agglutination Reactions of the Morgan Bacillus No. 1, J. Exper. M., Balt. **29:** 531-536, 1919.

B. pyocyaneus,[37] *Streptococcus fecalis*[38, 39] and *B. proteus*[3, 39, 40] can be similarly discounted as etiologic agents in diarrhea, for they are to be found in the stools of many normal children.

The theory[41, 11] advanced during the last century by French and German writers that infantile diarrhea was caused by highly virulent colon bacilli has died a natural death from lack of confirmation.

EPIDEMIOLOGY AND PREVENTION

Our studies in Birmingham revealed in many cases a well-defined history of contact infection from a neighbor's child or from an adult with a mild diarrhea; in several instances in Baltimore two cases occurred in the same house.

An examination of the source of the milk supply showed that the dairy could not be the origin of the infection. Studies in adult dysentery have shown that although widespread epidemics may occasionally be water-borne, dysentery bacilli are rarely recovered from central milk or water supplies.[24, 42, 54] It is sometimes maintained that inasmuch as the majority

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37. Calmette (Cochin-China), Lartigau (U. S. A.), Adami (Canada): "B. pyocyaneus as the Cause of Dysentery." In: A. Castellani and A. J. Chalmers, Manual of Tropical Medicine, 3d Ed., Lond., Baillière, Tindall & Cox, p. 1841, 1919. Escherich (Austria): In: M. Pfaundler and A. Schlossmann. The Diseases of Children, Eng. transl. ed. by H. L. K. Shaw and L. La Fétra. Philadelphia and London, J. B. Lippincott Co. **3**: 298, 1908.

38. Logan, W. R.: The Intestinal Flora of Infants and Young Children, J. Path. & Bacteriol., Cambridge **18**: 527-551, 1913-1914. Bravo and Frias: Acute Colitis in Children, Siglo méd., Madrid **66**: 1025, 1919. Oppenheim, C. J.: The Human Fecal Streptococci, J. Infect. Dis., Chicago **26**: 117-129, 1920.

39. Glynn, E. E.: The Relation Between Bacillus Enteritidis Sporogenes of Klein and Diarrhea, Thompson Yates Lab. Rep., Liverpool **3**: 131-150, 1901.

40. Booker, W. D.: A Bacteriological and Anatomical Study of the Summer Diarrheas of Infants, Johns Hopkins Hosp. Rep., Balt. **6**: 159-258, 1897. Tsiklinsky, Mlle.: Contribution a l'étiologie des Diarrhées des Nourrissons, Ann. de l'Inst. Pasteur, Par. **31**: 517-536, 1917.

41. Rossi Doria, Escherich, Finkelstein, Gilbert, Girod, Lesage and Macaigne. In: Pfaundler and Schlossmann. The Diseases of Children, Eng. transl. ed. by H. L. K. Shaw and L. La Fétra, Philadelphia and London, J. B. Lippincott Co. **3**: 295-297, 1908.

42. Shiga, K.: Bacillary Dysentery. In: Mod. Med., 2d Ed. (Osler & McCrae), Philadelphia and New York **1**: 770-774, 1913. Buchanan, G. S.: Study of an Outbreak of Bacillary Dysentery, Lancet, Lond. **2**: 166-168, 1918. Stookey, G. E.: An Epidemic of Water-Borne Dysentery, J. Infect. Dis., Chicago **25**: 331-334, 1919. Another Water Supply "Accident" (Current Comment), J. A. M. A., Chicago **74**: 606-607, 1920.

of the children suffering from dysentery are of the crawling age, the disease may be acquired through the child's fingers becoming soiled from the floor. It would appear in our series that the infection of the milk in the individual household by flies or the mother's fingers is also a possible explanation of its spread.

That the boiling of milk and milk mixtures before feeding has a most important influence on the prevention of diarrheal diseases has always been suspected and Knox and Powers [43] have found that the use of boiled milk mixtures in boiled containers has greatly reduced the incidence of dysentery among the children whose feedings have been supervised by the Babies Milk Fund Association of Baltimore.

That flies have been the means of carrying dysenteric infection has been clearly shown by the studies of Graham Smith [44] and others.[45] The epidemiology of the outbreaks of dysentery in Salonika [24] during the war has proven beyond a doubt that flies were offending agents during the summer months.

The curve (see Table 4) showing the data of onset of our cases in Baltimore demonstrates that the majority of the cases occur during the height of the fly season. Table 4 also shows the fallacy of calling dysentery in children "summer diarrhea," for although the greatest incidence was in August, yet cases occurred regularly during the winter months.

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43. Knox, J. H. M., Jr., and Powers, G. F.: Report of the Babies Milk Fund Association of Baltimore, Transactions Am. Child Hygiene Assn., Albany, N. Y. **10:** 275-279.

44. Graham-Smith, G. S.: Bacilli Isolated from Flies in Normal Surroundings and in Surroundings Associated with Epidemic Diarrhea, Loc. Gov. Board Reports, 1911-12, **41,** Appendix B, No. 4, p. 304.

45. Bahr, P. H.: Report of the London School of Tropical Medicine on Investigations on Dysentery in Fiji During the Year 1910, London, 1912, Witherby & Co., 77 pp., 8°. Suppl. No. 2, J. Lond. School Trop. M. Cox, G. L.; Lewis, F. C., and Glynn, E. E.: The Number and Varieties of Bacteria Carried by the Common House Fly in Sanitary and Insanitary City Areas, J. Hyg., Cambridge, **12:** 290-319, 1912. Woodcock, H. M.: Note on the Relative Proportions of Amebic and Bacillary Dysentery Among the Troops of the Egyptian Expeditionary Force During the Season of 1917; Together with Some Remarks on the Question of Cyto-Diagnosis, J. Roy. Army Med. Corps, London **34:** 121-130, 1920. Sweet, E. A.: The Transmission of Disease by Flies, Wash., 1916, 20 pp., 8°. Suppl. No. 29 of Pub. Health Rep.

Conradi in Metz[46] and Ohno in Japan[47] demonstrated *B. dysenteriae* in mild cases and normal individuals who had been in contact with dysentery patients during the winter months, so the probabilities are, that dysentery bacilli, which live with difficulty outside of the human body, weather the winter by means of chronic or mild cases or carriers and in the spring are ready for wider dissemination by flies.

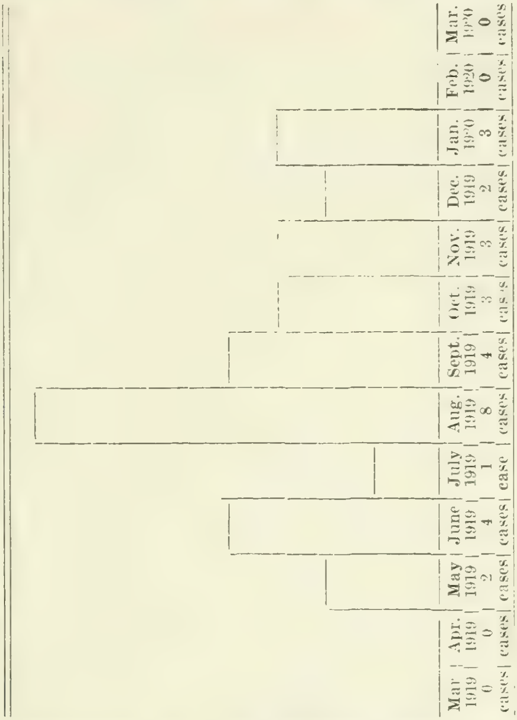

TABLE 4.—INCIDENCE OF BALTIMORE DYSENTERY CASES

The lesson to be drawn from these epidemiologic studies is obvious. Children should be breast fed or given only boiled milk in boiled containers. Children and milk should be protected from flies. When cases occur, the stools should be completely covered so that flies do not have access. Almost invariably mothers will save specimens, for the visit of the doctor, either wrapped in a newspaper or in an imperfectly covered receptacle. Those engaged in caring for a patient with dysentery should not prepare food for other children. These recommendations seem self evident but they are necessary and are difficult to enforce rigidly.

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[46] Conradi, H.: Ueber eine Kontaktepidemie von Ruhr in der Umgegend von Metz, Festschr. z. 60. Geburtst. v. Robert Koch, Jena, 1903, 551-570.

[47] Ohno: Carriers of B. Dysenteriae. In: Mod. Med., 2 ed. (Osler & McCrae), Philadelphia and New York **1**: 771, 1913.

Whether inoculations with dysentery vaccines will prove of value in children is problematical Heretofore the severe reactions to these vaccines have prevented their use except in the face of a grave epidemic,[48] but the recent work of Graeme Gibson[49] in the British Army would seem to indicate that the reactions may be eliminated without impairing the value of the protection. Wholesale inoculations will probably never be necessary but eventually it may prove of value in cities that have a high incidence of infantile dysentery.

At present the treatment of this condition, as of typhoid fever, is merely palliative and does not seriously affect the mortality, yet the stamping out of this disease by suitable quarantine by health departments is an attractive possibility. This can only occur by recognizing that ileocolitis is caused by dysentery bacilli and reporting it as dysentery. Just as the clinical name of enteric fever was changed to typhoid and paratyphoid fever, so ileocolitis and infectious diarrhea should be renamed in accordance with their etiology as dysentery in children. This plea was made in 1903 by Knox[9] following Duval and Bassett's pioneer work, but the old names still persist. One great advan-

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48. Stitt, E. K.: The Diagnosis and Treatment of Tropical Diseases, 2 ed., Philadelphia, 1917, P. Blakiston's Son & Co., 534 pp., 12°. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, 3 ed., London, 1919, Baillière, Tindall & Cox, p. 1860.

49. Gibson, H. G.: A New Method of Preparation of a Vaccine Against Bacillary Dysentery Which Abolishes Severe Local Reaction; also Experiments with This Vaccine on Animals and Man, J. Roy. Army Med. Corps, London **28**: 615-657, 1917. Idem: Results Obtained from the Use of Anti-dysenteric Serovaccine in the Field, with Regard to the Reduction of Case Incidence, J. Roy. Army Med. Corps, London **30**: 476-485, 1918.

tage to be obtained from such a change of nomenclature would be the making of the disease reportable. In many of our cities dysentery is a reportable disease but ileocolitis attracts no official notice. Inasmuch as in our series of cases, bloody stools and a febrile onset are practically pathognomonic of dysentery, it would seem that the majority of cases can be recognized on clinical grounds and reported to the health authorities.

CONCLUSIONS

1. Over 80 per cent. of the acute cases of ileocolitis both in Baltimore and Birmingham were due to infection with *B. dysenteriae.*

2. *B. dysenteriae* (Flexner) is more prevalent than *B. dysenteriae* (Shiga) in Baltimore, Maryland, and Birmingham, Alabama.

3. Clinically as well as bacteriologically the Baltimore and Birmingham cases were identical.

4. *B. dysenteriae* (either Flexner or Shiga) was not found in the stools of sixty-three cases of simple diarrhea nor in those of 100 normal children.

5. The name ileocolitis should be changed to dysentery in children and the disease made reportable to the health authorities.

6. Dysentery (ileocolitis) is probably spread from the stools of one patient to the food and mouths of other children by flies and infected hands and not disseminated from a general dairy source.

7. Dysentery is less prevalent among children receiving breast milk or boiled milk and boiled milk mixtures in boiled containers.

8. The agglutination reactions of the patient's serum by standardized technic is of assistance in the diagnosis of dysentery.

9. *B. Morgan No. 1 B. welchii, B. pyocyaneus, B. proteus* and the *Streptococcus fecalis* are not the cause of dysentery (ileocolitis) or diarrhea.

APPENDIX

BACTERIOLOGIC AND SERIOLOGIC TECHNIC

Stool Cultures.—A loop full of bloody mucus (unwashed) was streaked first over the surface of one Endo plate,[*] and then a second Endo plate without flaming the loop. After eighteen hours' incubation at 37.5 C. the colorless colonies were fished into tubes of Russell's double sugar medium[50] (Andrade's[51] indicator). Those cultures that had colorless slants and red butts without gas after eighteen hours' incubation were subcultured in gelatin and seven Durham fermentation tubes of peptone water containing respectively 1 per cent. of lactose, dextrose, mannite, maltose, saccharose, dulcite and rhamnose (Bromcresolpurple indicator).[52]

The growth from the original culture (Russel's slant) was then emulsified in saline and agglutinated at 55 C. for four and one-half hours with polyvalent dysentery, Flexner and Shiga type, serums. Six- to eight-hour peptone water cultures were examined for motility and seven-day peptone water cultures were tested for indol by ether extraction and Ehrlich's reagent.[*]

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[*] Endo's medium employed was made as follows: Shred agar 20 grams; peptone (Difco) 10 grams; meat extract 5 grams; NaCl 5 grams; distilled or tap water 1,000 c.c. Boil; adjust reaction to p_H 7.8, filter and autoclave in 100 c.c. lots in flasks for 15 minutes at 15 pounds. Ten per cent. lactose in distilled water was similarly autoclaved in 10 c.c. lots in test tubes.

To pour plates: Add 0.25 c.c. of 10 per cent. basic fuchsin (in 95 per cent. alcohol) to a flask of 100 c.c. of melted agar. Dissolve 0.5 gm. of sodium sulphite in a test tube of 10 c.c. of 10 per cent. lactose and add the solution to the fuchsin agar. This will completely decolorize the agar. Pour into seven 100 mm. Petri dishes.

50. Russell, F. F.: The Isolation of Typhoid Bacilli from Urine and Feces with the Description of a New Double Sugar Medium, Jour. Med. Res. **25** (new series, **20**): 217, 1911-12.

51. Andrade, E.: Influence of Glycerin in Differentiating Certain Bacteria, J. Med. Research **14**: 551-556, 1905-6.

52. Clark, W. M., and Lubs, H. A.: A Substitute for Litmus for Use in Milk Cultures, J. Agric. Research **10**: 105, 1917.

[*] Glynn's method.[19]

One-half c.c. of ether was poured into the 7-day peptone water culture tube and the tube shaken. The supernatant ether was then pipeted off and stratfied on 1.0 c.c. of distilled water in an agglutination tube. 0.25 c.c. of Ehrlich's reagent (paradimethylamidobenzaldehyd, 4 parts; alcohol (95 per cent.) 380 parts; hydrochloric acid (conc.) 80 parts) was then allowed to flow down the inside of the tube. A purple layer at the ether-water junction within thirty minutes denoted the presence of indol. This method obviates the difficulty of differentiating true

The fermentation cultures were incubated at 37.5 C. and read at intervals, for over twenty-one days and the gelatin cultures were left at room temperature for twenty-eight days.

The necessity for prolonged incubation of fermentation cultures is shown by the fact that 12 per cent. of the cultures from children in this series and 29 per cent. of those from the series in troops in the A. E. F. were late lactose fermenters, that is, colorless colonies on Endo's medium that fermented lactose after the second day of incubation. However, all fermentations were practically complete by the fourteenth day and only very slight changes occurred during the remaining time of the twenty-one day incubation. It would seem that fourteen days' incubation for fermentation cultures is sufficient and not twenty-one days as often stated.

Agglutination.—Subsequent agglutination work to confirm the original tests was done with eighteen-hour peptone water cultures. For the diagnostic serums employed, I am indebted to the Rockefeller Institute for polyvalent dysentery, Flexner and Shiga, serums; to Dr. A. D. Gardner and the Medical Research Committee of England for typhoid, paratyphoid A and B, Shiga and Flexner V, W, X, Y and Z serums; to Capt. E. G. D. Murray, R. A. M. C. for Kruse E, Schmitz, Shiga and Flexner V, W, X, Y and Z serums; and to Prof. F. W. Andrews for Z serum.

Dreyer's [21, 17, 22] technic was used to test the patient's serum for agglutinins. Inasmuch as I used eleven different antigens in this work I had to adopt the following modification of this method. The patient's serum (at least 1.0 c.c. was required) was diluted ten times; 0.5 c.c. (or 10 drops) of this diluted serum was placed in each of eleven agglutination tubes; 0.5

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indol reactions from the subsidiary colors due to the peptone culture media. *Alternatively,* the Ehrlich reagent may be carefully added to the culture tube after shaking with ether so as to form a layer between the ether and the culture medium. The pseudo-indol reactions that occasionally are noted in this latter method may be distinguished by adding 0.5 c.c. of chloroform, for the true rosindol color is soluble in chloroform.

c.c. (or 10 drops) of a formalized standard agglutinable culture of each of the following organisms was placed in one of the eleven tubes; *B. typhosus, B. paratyphosus A, B. Paratyphosus B, B. shigae, B. dysenteriae* (Flexner), English types V, W, X, Y and Z, *B. dysenteriae* (Kruse E) (or *B. dispar*) and *B. dysenteriae* (Schmitz) (or *B. ambiguus*).

These tubes were incubated in a water bath at 55 C. for four and one-half hours. (Agglutination reactions with *B. dysenteriae* require longer incubation than those with *B. typhosus.* Four and one-half hours at 55 C. is the minimum time.) If agglutination was positive for any of these eleven cultures, tests were set up according to Dreyer's directions using only those standard agglutinable cultures for which there had been agglutinins in the preliminary test.

SOAPS IN RELATION TO THEIR USE FOR HAND WASHING *

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(From The Journal A. M. A., July 31, 1920, p. 302)

The public is asked to buy many things of a fraudulent nature, or concerning which fraudulent claims are made by the manufacturers or their agents. We are all familiar with the history of foods in the last twenty years and the great advance that has occurred since the passage of the Food and Drugs Act in 1906, in protecting the guillible public. This law and various amendments made an attempt to regulate a far greater evil than adulterated foods, i. e., the ubiquitous patent medicines. At the present time a manufacturer must not make "false or misleading" statements concerning the composition of his medicine and must not make curative claims which are "false or fraudulent"; but this applies only to the package in which the medicine is sold and not to circulars or newspaper advertisements apart from the package. He must also declare the presence and amount of eleven specified drugs. Closely allied to the "patent medicines" are the varieties of "healing," "antiseptic" and "germicidal" soaps, accompanied too often with claims of their value which to my mind have been neither proved nor disproved. I will cite two instances from the soaps with which we have been working for the last two years.

1. "May be used as a general antiseptic for wound cleansing, for washing sores, ulcers, *including cancerous infections* [italics in these quotations are mine].

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* From the Department of Hygiene and Bacteriology, University of Chicago.

* I have been aided in this work by Mrs. E. O. Jordan, Mr. J. E. Gordon, Mr. T. T. Crooks and Mr. H. M. Weeter, to whom thanks are due.

* This investigation was made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

. . . Useful for offensive odors about the body, *for bathing in contagion* and for many diseases of the skin."

2. "*Chemical* tests have shown that bacteria and germ life exist on ordinary soap, but that no germ life and bacteria can exist on . . . Not only will the body itself remain sterile but when rubbed over the body *in the usual manner of washing the skin is rendered sterile,* thus acting as a safeguard."

TABLE 1.—ORGANISMS FOUND BY CUMMING

Number	Condition	Organisms	Per Cent. Positive
340	Carrier	Streptococcus hemolyticus	37
40	Patients	Pneumococcus	15
95	Patients	B. diphtheriae	6
...	Carriers	Streptococcus viridans	100
78	Patients	B. tuberculosis	2

Other soaps are frankly advertised as antiseptic or germicidal, and many certainly convey to the buyer the impression that they are excellent means of killing the harmful germs which may be present on the skin when the soaps are used, as in the claim just quoted, "in the usual manner of washing."

There are several features connected with buying and using soaps. One is perhaps more economic than hygienic, i. e., should the public be asked to pay 30 or 35 cents for a special kind of soap unless it really possesses properties which make it more valuable than those selling for 12 or 7 cents? But far more important are the specific hygienic claims made for certain soaps. Are they frauds or do they really possess, for example, germicidal properties which really aid in the prevention of the spread of disease? Can the public depend on them or do they foster a false sense of security? It is certainly important to examine the basis on which these claims are made. Let us take, for example, the bactericidal properties. Whatever variety of tests have been made in the manufacturers' laboratories, for the buyer there is only one guide: the statement which is sometimes on the package concerning the "phenol coefficient" value. I think it may be admitted that soap solutions in *sufficient concentration* are antiseptic (not necessarily germicidal). In addition, many soaps contain such substances as cresols, which are known to be bactericides under the proper conditions. Hamilton[1] has stated that "no soap can be considered an effective disinfectant unless associated with some other more active agent." But this statement is thus qualified later in the same article: "Most disinfectants with the exception of potassium mercuric iodid add little to the efficiency of soap," referring to disinfecting properties. Is the "phenol coefficient" a proper means of measuring the value of soaps when used "in the usual manner of washing"? I think not, because of the great difference in conditions which exist in the laboratory tests and in the actual use of the soaps. We have no adequate tests and, indeed, a search of the literature reveals that almost no attempts at such tests have been made.

First, let us consider the necessity for removing bacteria from the surface of the body and particularly from the hands. Various considerations point to such a necessity and to the hands as vehicles in the transmission of infectious diseases. Transference of typhoid and paratyphoid bacilli from patients, convalescents and carriers to food can most easily be conceived as taking place by way of the hands. Material from the nose, as in colds, or from the mouth, as in diphtheria (or with diphtheria carriers), may be taken from the hands and conveyed to the hands of others either directly or by means of such objects as door handles or dishes or books, and thence will reach the mouth of a normal person and may infect him. Cumming[2] believes that the major part of the transmission of respiratory diseases is due to hand infection. His conclusions are drawn from such

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1. Hamilton, H. C.: Am. J. Pub. Health **7**: 282 (March) 1917.
2. Lynch, C., and Cumming, J. G.: Am. J. Pub. Health **9**: 25 (Jan.) 1919. Cumming, J. G.: Ibid. **9**: 414 (June) 1919.

data as these: Of groups comprising more than 66,000 officers and men in the army, about half washed their mess-kits in tepid water in barrels, the common property of each organization. The other half used table ware. Among the first group the influenza rate was 252 per thousand, in the second group only 51.1 per thousand. These results are explained by Cumming as due to the conveyance of the virus to the tepid water by the hands, the germs then being picked up on the hands of another individual and hence passing to the mouth either directly or indirectly. In another study[3] on the incidence of pneumonia in institutions, it was found that this incidence was three times as high where hand-washed eating utensils were used than in those in which there were machine washers. Whether or not the interpretation of these results is wholly correct, the figures certainly serve to indicate the importance of the hands as a possible means of transmitting disease.

If the hands are so important, it ought to be possible to find on them pathogenic bacteria or associated organisms. Many studies have been made on the flora of the skin, but most of them were concerned with normal forms, as staphylococci, rather than with those foreign organisms which are temporarily present on the outer surface and would be most easily removed. In attempting to establish experimentally the possibility of typhoid infection by way of the hands, Winslow,[4] more than fifteen years ago, made a search for *B. coli* on the hands of 111 individuals and found typical organisms present in ten instances. Cumming,[5] in the course of the experiments already referred to, found the organisms named in Table 1.

Weaver[6] has recently reported results obtained on hospital attendants in which two organisms were sought: *B. diphtheriae* and *Streptococcus hemolyticus*,

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[3] Cumming, J. G.: Am. J. Pub. Health **9**: 849 (Nov.) 1919.

[4] Winslow: J. M. Res. **10**: 463-471, 1903-1904.

[5] Cumming, J. G.: Am. J. Pub. Health **9**: 417 (June) 1919.

[6] Weaver, G. H., and Murchie, J. T.: Contamination of the Hands and Other Objects in the Spread of Diphtheria, J. A. M. A. **73**: 1921 (Dec. 27) 1919.

Barron and Bigelow[7] found *B. diphtheriae* in 16.1 per cent. of cultures from the hands of diphtheria patients and carriers who were not masked. Some years ago, Cummins[8] reported the isolation of typhoid bacilli from the hands of carriers. Since not all of the organisms mentioned in these experiments can be regarded as part of the normal flora of the hands, and were obtained by swabbing (Weaver) or washing in sterile water (Winslow), it is easy to see that with sufficient care of the hands, conveyance of infection could doubtless be decreased. This care, of course, consists mainly in adequate hand washing.

TABLE 2.—RESULTS REPORTED BY WEAVER

Number of Persons	Number of Cultures	Status of Persons	Percentage Positive	
			B. Diphtheriae	Streptococcus Hemolyticus
45	268	Pupil nurses	3.0	9.3
4	51	Graduate nurses	0.0	2.0
3	45	Interns	6.7	15.6
4	64	Diet maids	0.0	3.1
1	18	Orderly	0.0	22.0
		Laundry and office help	0.0	0.0

TABLE 3.—BACTERIA REMOVED FROM THE HANDS

Soap	Number of Tests	Wash Water	First Rinse Water	Second Rinse Water	Total
A	26	1,600,000	1,060,000	865,000	3,605,000
B	22	1,670,000	2,270,000	1,920,000	5,860,000*
C	18	79,000	73,500	149,000	201,500
D	13	790,000	420,000	520,000	1,730,000
E	1	19,500	24,500	†	
F	2	122,000	133,000	49,000	304,000
G	4	14,000	325,000	105,000	454,000
H	2	19,000	880,000	904,000	1,803,000

* This series contained one very high count.

It is of some importance, therefore, to know the value of various soaps in removing bacteria, in the destruction of bacteria removed and in killing any bacteria remaining on the hands. It seems to us that this could best be done by studies on hands as we found them rather than by artificially added foreign organisms. The literature of soaps is enormous. From a bacterial standpoint, the disinfecting properties of soap are of particular interest; but nearly all reports found deal either with the chemical constituents of soaps or with determinations of germicidal value of soap solutions according to methods comparable to those used in determining the phenol coefficient,[9] which, as has already been pointed out, are not done under conditions similar to those of actual hand washing. The only experiments of the latter kind are those reported in 1899 by Symes.[10] He tried the usual tests on cultures of bacteria in suspension, and concluded that "for practical purposes most of the so-called disinfectant soaps have no value, but in the combination of biniodid of mercury with soap we have a useful means of disinfecting hands, instruments, etc." Symes also tried hand washing experiments, but did not succeed in sterilizing the hands by this method even with mercuric iodid soap. No details of these experiments are given.

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[7] Barron, M., and Bigelow: J. Infect. Dis. **25**: 58 (July) 1919.

[8] Cummins, cited by Richardson: Bull. State Board of Health of Massachusetts **3**: 129, 1914.

In our experiments we have tried to determine four things: First, the relative efficiency of various soaps in removing bacteria from the hands. For this a standard procedure was devised and a large number of tests were made, since the comparison of results from single instances not made in any uniform way would be valueless. Second, the relative germicidal power of the soap solutions obtained in washing toward bacteria removed from the hands. Third, the bacterial condition of the hands after washing, i. e., whether sterile or not. Fourth, the effect of the soap, if any, remaining on the hands, comparing the action of different soaps in this respect.

In the experiments we used in all twelve soaps, varying from the cheapest toilet soaps to the expensive so-called antiseptic and germicidal soaps. These were picked at random from those on sale at retail drug stores. The experiments were made during various seasons of the year on students and members of the staff of the laboratory who were called from their regular work without previous warning. No seasonal differences were noted. In all the work the hands were washed in sterile distilled water at 40 C. in sterile pans. The number of bacteria was determined by plating on nutrient agar (reaction + 1 to phenolphthalein), and the plates were counted after twenty-four hours' incubation at 37 C. In order to work out some standard procedure, a long series of preliminary experiments was made in which conditions and numbers of washings and rinsings were varied. For example, it was found that wiping the hands on a sterile towel between washing and rinsing or between two rinsings resulted in an increased removal of bacteria in the final rinse. The use of a towel was therefore abandoned. Tests were also made to determine the maximum number of bacteria which could be removed by successive washings and rinsings. This number varied from 24,000 to 7,000,000 in six tests with six successive washings and rinsings. The average number of bacteria found in the last rinse water was 232,000—in one case 745,000. We can therefore conclude that it is impossible to obtain sterile hands in the ordinary process of washing. These results were confirmed by later work, done, however, for a somewhat different purpose. The procedure finally adopted was as follows: Hands are washed by the subject for one minute with soap in 500 c.c. of sterile distilled water at 40 C. in a sterile pan. According to trials, the time, one minute, is rather longer than is customarily spent in washing. The hands are immediately rinsed in sterile distilled water under the same conditions, and this rinsing is repeated. The wash water and rinse waters are then immediately plated to determine their bacterial content. Table 3 gives the average total bacteria removed from the hands according to the foregoing procedure. The variation in the number of bacteria in the different tests was tremendous. In one series of twelve tests with the same soap, the lowest number obtained in the wash water was 60,000, and the highest, 8,445,000.

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[9] Work of this kind is reported by Konradi: Arch. f. Hyg. **44**: 101-112, 1902. Rodet: Compt. rend. Soc. de biol. **63**: 264-266, 1905. Rasp: Ztschr. f. Hyg. u. Infektionskrankh. **58**: 45-63, 1907.

[10] Symes: Bristol M. & Chir. J. **17**: 193-197, 1899.

Of these eight soaps, A and B are well known toilet soaps, D is a green soap such as is used in hospitals, and the others are special soaps supposed to have particular power with respect to killing bacteria. The ordinary toilet soaps appear to have removed the greatest number of bacteria in the wash water, although the differences are not so marked in the rinse waters and totals. There are two explanations. One is that the special soaps have killed the bacteria or at least inhibited their growth so that they do not appear on the plates, and the other is that the ordinary toilet soaps actually have a greater cleansing power. It is certain that, with the soaps we used, a good lather is more easily obtained with the latter. It appears also that the larger number of bacteria in the first rinse water from the special soaps, in comparison with the number obtained in the wash water, might be explained by the difference in cleansing power, and leads us to doubt the germicidal action. However, these points needed further investigation.

TABLE 4.—BACTERIAL COUNTS

Number	Original Wash	1 Hour	3 Hours	5 Hours
1	1,380,000	2,460,000	1,360,000	1,385,000
2	179,000	143,000	136,000	137,000
3	560,000	181,000	153,000	168,000
4	74,000	71,000	82,000	108,000
5	350,000	1,090,000	110,000	163,000
6	35,000	0	0	0
7	0	19,500	7,500	0
8	24,600	36,000	48,000	4,500
9	2,000	4,500	3,500	2,000
10	23,000	17,500	56,500	19,000

The possible disinfecting or antiseptic properties of the special soaps were tested in two ways. The first method consisted in allowing the wash water to stand protected and the bacterial content determined after varying periods of time. This ought to give an indication as to the germicidal action of these soap solutions toward the bacteria found on the hands. Table 4 gives the bacterial counts as obtained from the original wash water and after this has stood for one, three and five hours. Experiments 1 to 5 are with ordinary toilet soaps; Experiments 6 to 10, with special soaps. Experiments 6 and 7 are with the same soap. These results show that for practical purposes the germicidal and antiseptic powers of the special soaps as well as of ordinary toilet soaps, in solutions as obtained in hand washing, are too small to be of any use. With two soaps containing mercuric iodid (probably potassium mercuric iodid, as this is the soluble compound), we were unable to obtain growths on our plates in twenty-four hours in all instances, either from the wash water immediately after use or after long standing. If the agar plates were allowed to incubate for forty-eight hours, growth was invariably obtained. These soaps apparently exhibited, particularly the one containing the larger amount of mercuric iodid, some germicidal and certainly marked antiseptic properties. An instance of growth after increased incubation is given in Table 5. The concentration of soap in the agar plates carried over from the wash water was about 0.05 per cent., in those from the rinse water about 0.001 per cent. This would mean that with 2 per cent. of mercuric iodid in the soap, 0.002 per cent. of mercuric iodid was sufficient to retard bacterial growth.

TABLE 5.—GROWTH AFTER INCREASED INCUBATION

	24 Hour Incubation	48 Hour Incubation
Wash water..................	0	19,500
Rinse water..................	0	24,500

That the soap solutions may fairly be compared was shown by determinations of the amount of soap actually used in washing, which, although different persons and different soaps were used, gave figures within reasonably narrow limits. The amount of soap in solution was determined by titrating with standard acid and reading from a plot the amount of soap corresponding to the titer. The plots were made by weighing out various quantities of the soap as bought, dissolving in water and titrating. Straight lines (within the limits tested) were obtained in every case, but the slope of the line varied with the kind of soap. The amount of soap used in hand washing under the conditions previously described varied in twelve tests between 2 and 5.1 gm., averaging 2.8 gm. This corresponds to a concentration of 0.5 per cent.

TABLE 6.—BACTERIAL CONTENT WITH RINSE WATER AFTER DRYING *

	Without Drying	After Drying
	580,000	17,500
	675,000	64,000
	1,760,000	238,000
	12,000	87,500
	17,000	52,500
	1,110,000	260,000
	144,000	30,000
	12,500	47,500
	72,500	23,800
	220,000	450,000
	46,000	2,630,000
		25,000
		3,000,000
Average	421,000	496,000

* The figures, standing opposite in the columns have no significance.

But in washing, the hands not only come in contact with the soap solution but also are rubbed with lather in which the concentration of soap is much greater. Furthermore, a small amount of the soap or its ingredients may be left on the hands. In order to get the effect of these factors, the hands were allowed to dry for five minutes after washing and were then rinsed in running sterilized distilled water, and the number of bacteria washed off was determined. This was done with a series of ten soaps all of which were supposed to possess antiseptic or germicidal qualities. In Table 6 the first column gives the bacterial content of the first rinse water from a series of experiments done as previously described, i. e., the hands were rinsed immediately after washing. The second column gives the figures from a series in which the hands were allowed to dry five minutes before rinsing.

These figures demonstrate that the soap remaining on the hands after washing has no germicidal action. The difference in the average figures is well within the limits of error of the work.

In this connection the amount of soap in the rinse water was determined as an index of the amount of soap left on the hands after washing. This proved to be about 2 per cent. of the amount of soap actually used or, on the average, 0.05 gm.

The same difficulty in obtaining growths of bacteria with soaps containing mercuric iodid was observed in these experiments as in the previous ones, and such growths (except in one instance) were obtained only after forty-eight hours' incubation. These soaps again exhibited distinct inhibitive properties.

CONCLUSIONS

Sterile hands are not obtained in the ordinary process of hand washing. More bacteria were found to be removed by the ordinary toilet soaps than by the special soaps. In other words, the cleansing properties of a soap are more important than its "germicidal" or "antiseptic" constituents.

The soap solutions obtained in hand washing are of no practical germicidal or antiseptic value.

The soap left on the hands after washing has no germicidal action.

In the whole process of hand washing done in the usual manner, the special so-called "germicidal" or "antiseptic" soaps exhibit none of these properties. Therefore, these terms are not proper to use in connection with soaps.

Finally, since the hands may serve as a medium for the conveyance of bacteria in infectious diseases, it is important to remove these bacteria; and this may be done by the ordinary toilet soaps as effectively, if not more so, as by the special brands of so-called "antiseptic' 'or "germicidal" soaps.

THE INFLUENCE OF REACTION ON THE PRECIPITATION OF PROTEINS BY TANNIN *

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From the Journal of Pharmacology and Experimental Therapeutics, August, 1920, p. 49

INTRODUCTION

The effects of typical astringents depend upon the precipitation of protein. In the case of tannins, the precipitation is greatly influenced by the reaction (H-ion concentration). This is particularly important when tannin drugs are administered internally, to produce local effects on the ailmentary canal, with its varying reactions.

The data in the literature are scanty and rather confusing, since they were gathered for the most part before the distinction between actual reaction and titrable reaction was clearly realized. The only investigation which takes this properly into account was that of Hanzlik (1915); and as this was made for another purpose, it dealt with dilutions that cannot be safely transferred to the ordinary conditions of astringent action.

Summary of Qualitative Statements as to Reaction on Tannin-Protein Precipitation. — The following seems to be a fair review of the statements of the literature.

Proteins, including albumin, glue, blood, peptones and pepsin, are precipitated by tannin, in neutral or faintly alkaline, or very feebly acid media only; these precipitates being redissolved by the addition of acids (even acetic) or strong alkalies (hydroxids, carbon-

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ates, borax), but not by weaker alkalies (sodium bicarbonate or disodium phosphate). They are also soluble in excess of protein.

Discordant statements are made as to "alkali-tannate." This is, tannin neutralized by sodium bicarbonate; and is probably of inconstant composition and therefore of inconstant reaction. According to some, the watery solutions do not precipitate proteins, but are nevertheless astringent to tissues. (Heinz, 1889; Lewin, 1904.) This only means that they become precipitant as soon as their excess of alkali has been absorbed by the tissues, and when, in other words, they have reached the H-ion concentration of the tissues.

Similarly, Gebhardt, 1899, found that all alkaline tannin solutions precipitate proteins when used as a "ring" test; i. e., at the point in the test-tube where the suitable H-ion concentration is reached.

The re-solution of the tannins in excess of protein is presumably due to a similar cause; i. e., an unsuitable reaction of albumin or blood; although further work is desirable.

Rost, 1897, believed that precipitation of protein occurs only in faintly acid reaction. Dixon conceived the precipitation as due to the acid radical (H-ion concentration?) of the tannin. Both statements would imply that tannin could not precipitate in the presence of a neutral or feebly alkaline buffer solution.

Incidentally, tannin precipitates also nonprotein colloids, for instance, starch.

Direct Determination of H-ion Concentration on Tannin-Protein Precipitation.—The only data that have been published are those of Hanzlik, 1915, in his investigation of the relation of alkaloidal precipitants to the isoelectric point of serum. This is the reaction at which the positive and negative charges held by the protein ions are about equal, and where there is a maximum of neutral particles of proteins (Michaelis). It lies about p_H 4.74. The precipitation by ethyl alcohol reaches its maximum at this point.

Hanzlik used horse-serum and glutin, dialyzed for five to six weeks, i. e., practically salt free. The experiments were made by adding one drop of 1 per cent. tannin, and one drop of 0.05 per cent. serum or 0.5 per cent. glutin to 2 c.c. of buffer-mixture. The final mixture therefore contained about 0.017 per cent. serum and 0.02 per cent of tannin.

With these conditions, precipitation appeared with p_H 4.14; reached its maximum at p_H 4.74; and was absent with p_H 5.35.

The maximum precipitation therefore occurred at the isoelectric point, and at a distinctly acid reaction. Other "alkaloidal precipitants" behaved differently, but do not concern us in this connection.

Increase of the serum content to 1 per cent. shifted the optimal reaction slightly toward the alkaline side.

Neutral salts, such as chlorid or sulphocyanid had little effect on the precipitation.

OBJECTS OF PRESENT INVESTIGATION

The deficiencies and contradictions of the literature indicated the desirability of determining anew the optimum and threshold H-ion concentrations, using higher concentrations of tannin than those employed by Hanzlik. Krameria and catechu were also tried, as representing the "natural vegetable astringents." Gallic acid was tested, as our samples were somewhat precipitant to protein under suitable conditions.

Serum-protein, egg-albumin and "peptone" (Witte) were used, as representative of the proteins that the tannin would encounter in the tissues or contents of the ailmentary canal.

GENERAL METHOD OF EXPERIMENTATION

This consisted in mixing the tannin, and protein in the presence of a buffer solution of the desired reaction, adding the indicator, and then sufficient acid or alkali to secure the desired change in the indicator. It was aimed to cover a range between $p_H = 1$ to 10.

Three series of experiments were made, differing in the quantity of buffer added. They will be given in chronologic order, although Experiment 1 contained the least buffer, and is therefore the least accurate.

The percentage of the chemicals in the finished mixtures was as follows:

	Experiment 1	Experiment 2	Experiment 3
Serum, dried	1	¼	1/10
Albumin, moist	5	2½	1
Peptone	½	¼	1/10
Tannin	½	¼ and 1/16	⅓
Krameria	1½ (extract)	1¼ (root)	
Catechu	1¼		
Gallic acid	⅜	⅜	

Original Protein Solutions.—These were made up in 0.9 per cent. NaCl on the preceding afternoon and filtered during the night.

S = *Serum;* dried serum, 1 per cent. dissolved by trituration with the solvent, standing half an hour, and filtering through paper.

A = *Albumen;* 10 per cent. of fresh egg-white, squeezed with the solvent through cheese cloth, and filtered.

P = *Peptone;* Witte's dry: 1 per cent. warmed with the solvent and filtered.

Designation of effects: These are ordered as:

$$- = \text{no change}$$

$$\text{T} = \text{turbidity}\begin{cases}\text{sl} = \text{slight}\\ \text{st} = \text{strong}\end{cases}$$

$$+ = \text{precipitate}\begin{cases}\text{sl} = \text{slight}\\ \text{st} = \text{strong}\end{cases}$$

Method in Experiment 1.—The tannin was dissolved in 0.9 per cent. sodium chlorid solution, with the addition of sodium bicarbonate, of the percentage stated under each experiment. This was intended to act as a buffer.

Ten cubic centimeters of these solutions were mixed with 0.5 c.c. of indicator, the required amount of acid or alkali, and sufficient 0.9 per cent. NaCl to make

20 c.c. These solutions therefore contained one-half of the original quantity of tannin; 5 c.c. of the solutions were then mixed with 5 c.c. of the protein solutions. The final mixtures therefore contained one-fourth of the original tannin and one-half of the original proteins.

The buffer value is small, and the reaction is further liable to change by the final addition of the protein solutions.

Method in Experiment 2.—In this, the reagents were mixed in the presence of appropriate buffer solutions, and the p_H adjusted after the addition of the protein.

The following solutions were used.

Protein solutions. The same as in Experiment 1.

Protein buffer solutions. These were made by adding 1 volume of protein solution to 2 volumes of the buffer solutions, made up according to Sörensen-Michaelis (glycocoll; citrate; phosphate; borax). The indicators were added and if the tint differed from that of the original buffer-solution, N/10 HCl or 1 per cent. sodium carbonate were added, until the tints matched.

Astringent solutions. The following ere used; the solvent in all being 0.9 per cent. sodium chlorid.

Tannin, 1 per cent.; sodium bicarbonate, 0.5 per cent.

Tannin, 0.25 per cent.; sodium bicarbonate, 0.70 per cent.

Krameria extract, 5 per cent.; sodium bicarbonate, 0.5 per cent.

Gallic acid, 1.5 per cent.; sodium bicarbonate, 0.66 per cent.

Order of mixture. Five cubic centimeters of the astringent solution were added to 15 c.c. of the buffer-protein solutions.

Method of Experiment 3.—This differed from Experiment 2 only in having a lower ratio of protein to tannin, in a higher proportion of buffer.

The buffer-protein mixture was made with 10 c.c. of buffer solution and 1 c.c. of protein solution. In the test, 5 c.c. of tannin solution were added to the mixture.

SUMMARY OF RESULTS

These are presented as graphs, in Figures 1 and 2; the depth of the shading corresponding to the density of the precipitation. The symbols are those already explained. "S" stands for serum; "A" for albumin (egg-white) and "P" for Witte peptone.

The charts show that the relation of the tannin-protein precipitation to hydrogen-ion concentration varies somewhat with conditions; but is broadly similar. The outstanding features are as follows:

1. There is practically no difference in the behavior of serum, albumin, and peptone.

2. The maximum of precipitation is between p_H 2 and 5.

3. The extremes of reaction that permit precipitation by tannin lie between $p_H =$ less than 1 and $p_H = 8$ to 8.3. The acid threshold varies with the concentration of tannin. With 0.5 per cent. of tannin, the precipitation at $p_H = 1$ is still dense. With 0.33 to 0.25 per cent., there is moderate precipitation or strong turbidity at this reaction. With 0.06 per cent. of tannin, there is merely turbidity beyond $p_H = 2$.

4. Variation of the protein content of two and one-half times (Experiments 2 and 3) affected only the amount of precipitation, but not the relation of the reaction.

5. Krameria and catechu behave essentially like dilute tannin. The precipitation limits are the same.

6. Gallic acid has the same precipitation maximum as tannin; but the extremes lie between $p_H = 1$ to 2 and $p_H = 5$ to 6. Presumably, the precipitation was due to the admixture of a trace of tannin.

Comparison with Results of Hanzlik.—Hanzlik, working with $\frac{1}{50}$ per cent. tannin, found the maximum of precipitation at $p_H = 4.74$, and the extremes between 4.14 and 6.35. The differences conform with the tendency of dilution of tannin in my experiments; i.e., the shifting of the maximum toward the alkaline side, and the approach of the extremes in both directions.

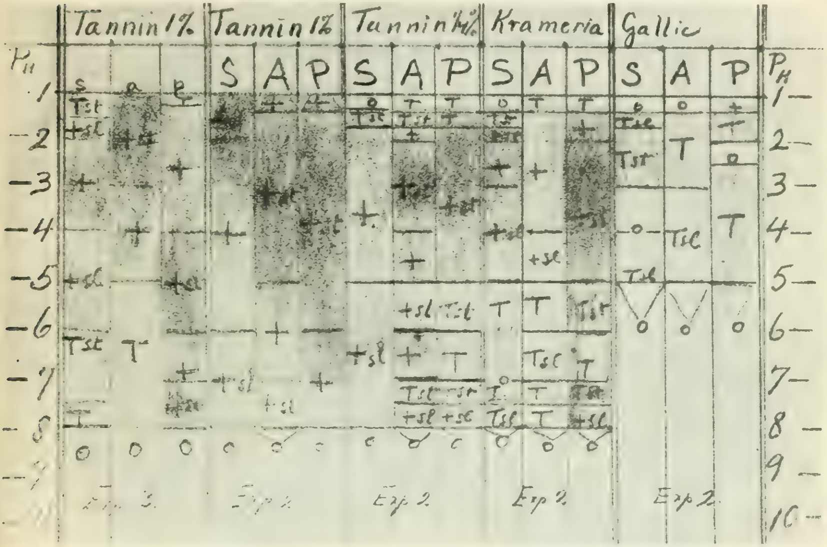

Fig. 1.—Effect of hydrogen-ion concentration on tannin precipitation. Experiments 2 and 3. *S* = serum; *A* = albumin; *P* = peptone. Experiment 3 contained a lower ratio of protein. The shading indicates the intensity of precipitation.

APPLICATION OF THE RESULTS TO THE DIGESTIVE TRACT

The results of these experiments render materially clearer the understanding of the possible action of tannins in the digestive tract.

Mouth. The p_H of saliva is quoted by Michaelis as 6.9. At this reaction, tannin, krameria and catechu are distinctly precipitant. This agrees with the marked astringency of these precipitants in the mouth.

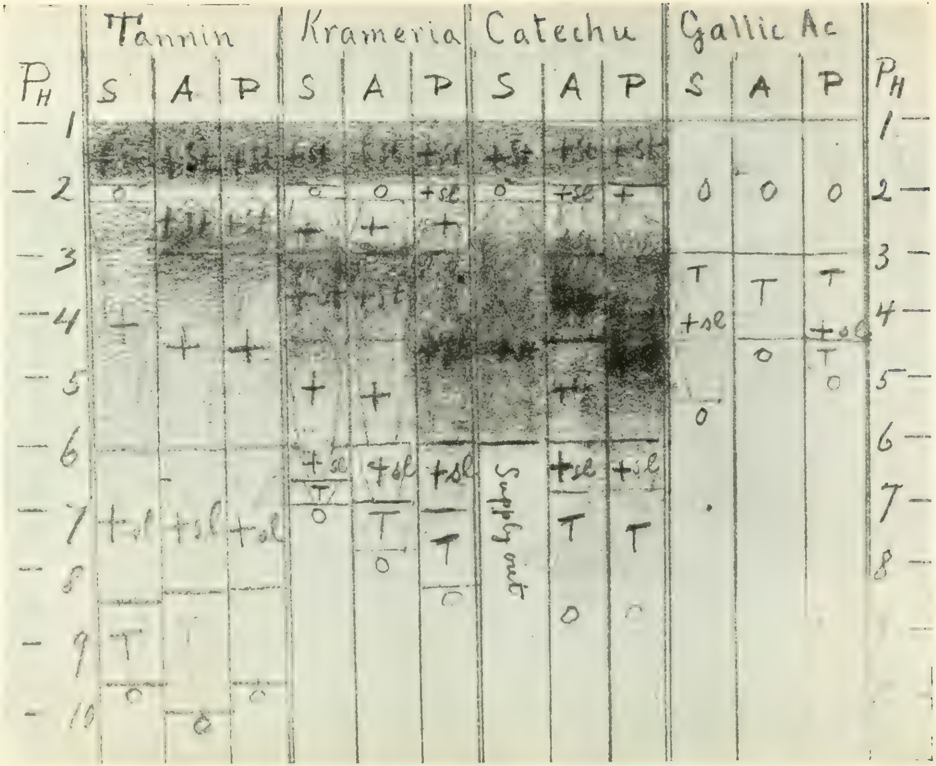

Fig. 2.—Effect of hydrogen-ion concentration on tannin precipitation.

Experiment 1. This is less reliable than Figure 1, since it contained less buffer. Nevertheless, it shows a general agreement. The range of the tannin is greater because the concentration of the tannin was higher than in Curve 1.

Stomach. The resting empty stomach may be nearly neutral. During the first one and one-half to three hours after a meal, the acidity rises rapidly to $p_H = 2$ or even 1; depending on the individual. It then remains stationary until nearly all the food has left the stomach (McClendon, 1915).

The reaction of the stomach comes well within the limits suitable for the tannin-precipitation.

If tannin is taken without food, it would exert considerable astringent action on the neutral or slightly acid stomach. If it is taken with food there would also be some astringency in the gastric mucosa; but the precipitation would concern mainly the proteins of the food. The precipitation would increase at first with the increasing acidity of the gastric contents; but before the end of the gastric digestion is reached, a part of the precipitate would be redissolved; not because of the formation of "peptones" for these are equally precipitable, but because of the excessively acid reaction.

The chyme as it passes the pylorus would therefore almost always contain tannin in three forms: as tannin-protein, precipitate; free tannin adsorbed into the precipitate; and tannin-proteinate held in solution by the acidity of the chyme. The adsorbtion is doubtless an important factor; for it is difficult to remove the excess tannin from tannin protein precipitates by simple washings.

Duodenum. The reaction of the duodenal contents is the resultant of the neutralization of the acid chyme by the slightly alkaline intestinal and pancreatic juice and bile. The approximate p_H of these is stated as: intestinal juice, 8 (Michaelis); pancreatic juice, 8.3 (Michaelis); bile, 7.8 (Okada). The extreme alkalinity would therefore be about 8 to 8.3, i. e., just about the limit of tannin-precipitation.

The actual p_H of the duodenal contents, however, is much more acid. Myers and McClendon, 1920, found the usual reaction in man to be between 3.2 and 5, when the precipitation would be optimal.

As the occurrence of astringent effects depends on whether the reaction in the duodenum is more or less favorable to precipitation than that of the gastric chyme, it is evident that astringency would occur whilst the over-acid chyme was in process of neutralization; but that it would probably cease when the acidity falls below $p_H = 7$.

Small intestine. Long and Fenger, 1917, found the reaction of the intestinal contents slightly acid throughout the small intestine; the acidity decreasing somewhat with the distance from the pylorus. In the human jejunum, it ranged from $p_H = 3.8$ to 7.8, mean 5.8; in the upper third of the small intestine in hogs, it ranged from 6.6 to 7.4, mean 6.7; in the middle third, 6.5 to 7.6, mean 6.9; in the lower third, 6.4 to 8.1, mean 7.

Similar figures were obtained in calves, lambs and dogs. They have been confirmed by McClendon et al., 1920.

These reactions fall well within the precipitation limits of tannin with protein. However, the reaction becomes progressively less favorable from the duodenum onward. There would consequently be no astringency beyond the duodenum, except in proportion as new supplies of tannin become available by the complete cleavage of the proteins. This would liberate the tannin that had been bound or adsorbed by the protein. However, this tannin would be promptly fixed by the cells of the intestines. Astringent action could therefore extend only to that level of the intestines at which the cleavage of protein is practically completed.

This ordinarily would be long before the ileo-cecal valve is reached. Accordingly, it would be quite impossible to obtain astringent effects in the large intestine, unless peristalsis or protein cleavage are very abnormal, as for instance in cholera. This agrees with the observation that normally, the feces do not show any tannin after the administration of large doses (8 grams) of the drug. (Moerner, 1892.) The conditions with "insoluble" tannin are different, as will be discussed in another paper.

CONCLUSIONS

1. The precipitation of proteins by tannin depends on the hydrogen-ion concentration of the medium. With concentrations of tannin such as would be used for astringent effects (0.1 to 0.5 per cent.), precipitation is maximal between $p_H = 2$ to 5; with greater acidity or alkalinity, the precipitation becomes smaller. With alkalinity greater than $p_H = 8$ or 8.3 there is no precipitation, and therefore no astringency. The limits are somewhat more narrow for dilute than for concentration solutions of tannin.

2. The precipitation limits are essentially the same for native proteins (serum and albumin) and for "Witte peptone." They are therefore not materially affected by digestion unless this goes beyond the stage of albumoses and probably peptones.

3. Krameria and catechu have the same limits as ordinary tannin. Gallic acid produces only very slight precipitation, within the same limits as tannin, and probably due to contamination with this.

4. These data suggest the following as to astringency by tannic acid in the alimentary tract:

a. The resting empty stomach would permit precipitation of the mucosa, and therefore astringency or irritation of the stomach. The tannin would thus be bound probably completely and prevented from acting on the intestine.

b. When taken with food there would be some astringent action but the precipitation would involve mainly the food proteins. These precipitates would also adsorb some of the tannin. The precipitation would at first increase with the acidity; but at the maximum acidity, a part would be redissolved.

c. The chyme, as it reached the duodenum, would contain tannin in the form of protein precipitates, adsorbed tannin, and redissolved tannin.

d. In the duodenum, the conditions would be favorable to further precipitation, and therefore to astringent action.

e. The reaction of the entire small intestines would permit precipitation, if any tannin were available. However, the reaction becomes progressively less favorable from the duodenum downward. Consequently, no further precipitation could occur after the duodenum or jejunum, except by the liberation of bound tannin. Such liberation occurs to some extent by the ultimate cleavage of the protein by which the tannin had been bound or adsorbed. However, this cannot extend only so far in the intestine as protein-cleavage extends; for the liberated tannin would be immediately bound by the intestinal mucosa.

f. It is therefore impossible that the astringent action should extend to the large intestines, unless peristalsis or protein cleavage are very abnormal.

g. Solutions of krameria or catechu would behave like tannin, and it is difficult to see how they could have much advantage. The crude drugs or solid extracts might possibly be superior, namely, if they dissolve only slowly in the intestines.

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A STUDY OF THE ANTISEPTIC ACTION OF CERTAIN LOCAL ANESTHETICS

I. ANTISEPTIC ACTION OF LOCAL ANESTHETICS AGAINST STAPHYLOCOCCUS AUREUS AND B. COLI *

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II. A STUDY OF THE ANTISEPTIC ACTION OF BENZYL ALCOHOL AND OTHER LOCAL ANESTHETICS AGAINST THE GONOCOCCUS †

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I. THE ANTISEPTIC ACTION OF LOCAL ANESTHETICS AGAINST STAPHYLOCOCCUS AUREUS AND BACTERIUM COLI

Although healing by first intention without infection is next to efficient anesthesia, the primary desideratum of every operation performed under local anesthesia, it is surprising to find that no investigation has been made concerning the antiseptic properties of local anesthetics. This may perhaps be due to the fact that the two most popular anesthetics, cocain and novocain, show nothing in their pharmacologic action nor in their chemical structure, which would lead one to suspect that they were in any way germicidal or antiseptic in their properties; indeed, the solutions of these

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anesthetics are well-known to become contaminated with bacteria and molds. With the introduction of newer drugs for local anesthesia, the interest in the subject however has been revived. Macht and Nelson[1] have called attention to the fact that benzyl alcohol, the new local anesthetic discovered by one of the authors (M.) exhibited a distinct germicidal action on some bacteria. This observation stimulated the authors to carry out the present investigation. In the present research, the authors have undertaken a study of the antiseptic germicidal properties of the most common local anesthetics in relation to some well-known bacteria. Such an inquiry was deemed desirable not only from a purely scientific point of view, but because also of a practical bearing, in at least two respects. In the first place, a local anesthetic which is also an antiseptic would tend to minimize the chances of infection after surgical operations. In the second place, such an anesthetic, when employed in certain regions or organs, would tend to destroy the bacterial flora in those locations. Thus, for instance, in ophthalmologic practice, such an anesthetic and antiseptic may be helpful in cases of infections of the eye. Again, and of still greater importance, a local anesthetic with antiseptic properties would be extremely desirable for use in urologic practice for instillation into the urethra, the bladder, or ureters.

METHODS

The following drugs were studied: cocain, novocain, stovain, alypin, holocain, alpha-eucain, beta-eucain, apothesin or the hydrochlorid of diethyl-amino-propyl cinnamate, and benzyl alcohol. All these drugs were dissolved in physiologic saline solution in various concentrations and their antiseptic action was studied on two micro-organisms, the *Staphylococcus aureus* and *B. coli.* Three methods of studying the problem were employed which are described below.

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[1] Macht and Nelson: Proc. Soc. Exp. Biol. and Med. **16**: 25, 1918.

Series 1.—In this series of experiments, different amounts of a 1 per cent. solution of the various drugs were introduced into 5 c.c. bouillon medium, so as to make mixtures of various concentrations of the anesthetics, ranging from 0.04 to 0.4 per cent. A platinum loopful of staphylococcus or colon cultures was quickly introduced into the media thus prepared, and the test tubes were put into the incubator for twenty-four hours. The growth of the germs in the anesthetic bouillon mixtures was then observed. At the same time control experiments were made with the same germs in the same bouillon medium but without the addition of drugs. The results of this series of experiments are tabulated in Table 1.

TABLE 1

Drug	Organism	0.2 c.c. (0.04 per Cent.)	0.5 c.c. (0.1 per Cent.)	1 c.c. (0.2 per Cent.)	2 c.c. (0.4 per Cent.)
Cocain	Staphylococcus	Abundant	Abundant	Moderate	Moderate
	B. coli	Abundant	Moderate	Moderate	Slight
Novocain	Staphylococcus	Abundant	Abundant	Moderate	Slight
	B. coli	Moderate	Moderate	Moderate	Slight
Alypin	Staphylococcus	Abundant	Moderate	Moderate	Slight
	B. coli	Moderate	Moderate	Moderate	Slight
Stovain	Staphylococcus	Moderate	Slight	Slight	Negative
	B. coli	Moderate	Moderate	Moderate	Moderate
Holocain	Staphylococcus	Slight	Slight	Negative	Negative
	B. coli	Moderate	Moderate	Slight	Negative
Apothesin	Staphylococcus	Abundant	Abundant	Abundant	Negative
	B. coli	Abundant	Moderate	Slight	Slight
Alpha eucain	Staphylococcus	Moderate	Moderate	Moderate	Slight
	B. coli	Moderate	Moderate	Slight	Negative
Beta eucain	Staphylococcus	Moderate	Negative	Negative	Negative
	B. coli	Slight	Negative	Negative	Negative
Benzyl alcohol	Staphylococcus	Abundant	Moderate	Negative	Negative
	B. coli	Moderate	Slight	Negative	Negative
Control	Staphylococcus	Abundant	Abundant	Abundant	Abundant
	B. coli	Abundant	Abundant	Abundant	Abundant

Series 2.—In this series of experiments a loopful of staphylococcus or colon culture was introduced into solutions of the drugs in different concentrations (ranging from 0.01 to 5 per cent). These suspensions were then put into the incubator for different periods of time, ranging from ten minutes to twenty-four

TABLE 2

Concentration of Drug, %	Kind of Organism	Exposure				
		10 Min.	30 Min.	1 Hr.	3 Hrs.	24 Hrs.
	Cocain					
0.1	Staphylococcus aureus	+++	+++	+++	+++	+++
	B. coli.................	+++	+++	+++	+++	+++
0.5	Staphylococcus aureus	+++	++−	+++	+++	+++
	B. coli.................	+++	+++	+++	+++	+++
1.0	Staphylococcus aureus	+++	+++	+++	+++	++
	B. coli.................	+++	+++	+++	++	—
5.0	Staphylococcus aureus	+++	+	++	+	—
	B. coli.................	+++	+++	+++	+++	—
	Novocain					
1.0	Staphylococcus aureus	+++	+++	+++	+++	+
	B. coli.................	+++	+++	+++	+++	++
5.0	Staphylococcus aureus	+++	+++	+++	+++	++
	B. coli.................	+++	++	+++	+++	++
	Holocain					
0.1	Staphylococcus aureus	+++	+++	+++	+++	+++
	B. coli.................	+++	+++	+++	+++	+++
0.5	Staphylococcus aureus	+++	+	+	+	—
	B. coli.................	+++	+++	++	++	—
1.0	Staphylococcus aureus	++	—	+	—	—
	B. coli.................	++	++	++		—
	Alpha eucain					
0.5	Staphylococcus aureus	+++	+++	+++	++	—
	B. coli.................	+++	+++	+++	+++	+
1.0	Staphylococcus aureus	+++	+++	+++	+++	—
	B. coli.................	+++	+++	+++	+++	—
	Beta eucain					
0.5	Staphylococcus aureus	+++	+++	+++	++	—
	B. coli.................	+++	+++	+++	+++	—
1.0	Staphylococcus aureus	+	++	—	+++	—
	B. coli.................	+++	++	++	+	—
	Stovain					
0.5	Staphylococcus aureus	+++	+++	+++	+++	—
	B. coli.................	+++	+++	+++	+++	—
1.0	Staphylococcus aureus	+++	+++	+	+	—
	B. coli.................	+++	+++	++	+	—
5.0	Staphylococcus aureus	+++	+++	+++	—	—
	B. coli.................	+++	++	—	—	—
	Apothesin					
0.5	Staphylococcus aureus	+++	+++	+++	—	—
	B. coli.................	+++	+++	+++	+++	—
1.0	Staphylococcus aureus	+	+	+	—	—
	B. coli.................	+++	+++	+++	+++	—
5.0	Staphylococcus aureus	—	—	—	—	—
	B. coli.................	—	—	—	—	—
	Alypin					
1.0	Staphylococcus aureus	+++	+++	+++	+++	+++
	B. coli.................	+++	+++	+++	+++	—
5.0	Staphylococcus aureus	—	—	+	—	—
	B. coli.................	—	—	+	—	—
	Benzyl alcohol					
0.5	Staphylococcus aureus	+++	+++	+++	+++	—
	B. coli.................	+++	+++	+++	—	—
1.0	Staphylococcus aureus	+++	+++	+++	++	—
	B. coli.................	+++	+++	+++	+++	—
3.0	Staphylococcus aureus	—	—	—	—	—
	B. coli.................	—	—	—	—	—
	Methyl alcohol					
5.0	Staphylococcusaureus	+++	+++	+++	+++	+++
	B. coli.................	+++	+++	+++	+++	+++
	Ethyl alcohol					
5.0	Staphylococcusaureus	+++	+++	+++	+++	+++
	B. coli.................	+++	+++	+++	+++	+++

hours. They were then taken out of the incubator and rapidly centrifugalized. The fluid was then poured off and the organisms were washed with saline solution and separated again by centrifuging. The bacteria were then taken up with a loop and cultured on agar media. After incubating for twenty-four hours, the culture media were examined in order to determine whether any growth took place or not. For control experiment germs from the same cultures were suspended in normal physiologic saline solution for different intervals of time, then centrifuged and planted on agar media and incubated for twenty-four hours. The results obtained in this series of experiments are shown in Table 2.

Series 3.—In this set of experiments, a number of local anesthetics were incorporated or mixed with agar media in proportions of 0.5 or 1 per cent. Staphylococcus and colon organisms were then planted on the media. The cultures were incubated for twenty-four hours and the growth or nongrowth of the organisms was then observed. For control experiment the same kind of organisms were cultured on plain agar media, without that mixture of drugs. The results of these experiments are shown in Table 3.

TABLE 3

Medium	Strength of Drug, per Cent.	Organism	Growth
Cocain agar	1	Staphylococcus aureus	Abundant
		B. coli	Abundant
Stovain agar	1	Staphylococcus aureus	Negative
		B. coli	Negative
Apothesin agar	1	Staphylococcus aureus	Negative
		B. coli	Negative
Alypin agar	1	Staphylococcus aureus	Negative
		B. coli	Negative
Benzyl alcohol agar	1	Staphylococcus aureus	Negative
		B. coli	Slight
Benzyl alcohol agar	3	Staphylococcus aureus	Negative
		B. coli	Negative

DISCUSSION

A study of the above tables will reveal the fact that while some of the substances studied exert practically no antiseptic or bactericidal action, others on the other hand exhibit quite distinct positive antiseptic and germicidal properties. The first series of experiments of course reveals more the antiseptic rather than the germicidal effect of the various drugs. In the second series, however, where the various organisms were exposed to the action of the various drugs for different periods of time and then washed, a failure to grow on agar afterwards points to a bactericidal or germicidal effect on the organisms. The third series of experiments is only interesting in connection with the first two series as corroborating the results obtained. Taken by itself, this group of experiments is of little value owing to the well-known objections to the use of impregnated culture media.

It is interesting to note that our two best-known and most widely used local anesthetics, namely cocain and novocain, are entirely devoid of any antiseptic action. On the other hand, it will be noted that a number of other well-known anesthetics possess or exhibit definite antiseptic and even germicidal effects. Thus, it will be noted that alypin possesses such properties but only in concentrations of 5 per cent. Slight antiseptic properties are also exhibited by holocain, stovain and the eucains, but only after long incubation periods. A study of Table 1, furthermore, reveals an interesting difference in the antiseptic properties between alpha-eucain and beta-eucain. It will be noted that the beta variety is more antiseptic in its action than the alpha variety. It is also interesting to note that the various antiseptic effects are not exhibited in the same degree in case of both of the organisms studied.

By far the most interesting drugs in connection with the present investigation are apothesin and benzyl alcohol. Both of these drugs exhibit quite a marked antiseptic action. It will be noted, however, that in Series 1, the antiseptic action of benzyl alcohol is more prominent. In Series 2, apothesin 5 per cent. proved to be germicidal as was also benzyl alcohol 3 per cent. Inasmuch as, however, apothesin is used only in solutions of 1 per cent. strength and certainly of not over 2 per cent. on the one hand, and the benzyl alcohol is most efficient without being in the least toxic in concentrations of 3 to 4 per cent., it will be seen that the latter drug is the most antiseptic as well as the most germicidal of the local anesthetics studied. It is especially interesting to note (Table 2) that neither methyl alcohol nor ethyl alcohol even in strengths of 5 per cent. excited any antiseptic effect.

The above interesting findings in regard to the antiseptic action of some local anesthetics made it desirable to test some of the more efficient ones in respect to their effects on some other micro-organism and more particularly in respect to their effects on the gonococcus. Studies on this subject have been made by one of the authors in association with Dr. E. O. Swartz, and the results were of sufficient interest to warrant a separate paper on the subject, which will be found to follow the present one.

CONCLUSIONS

1. The effects of a number of local anesthetics were studied on *Staphylococcus aureus* and *Bacterium coli* by three different methods.

2. It was found that some of the drugs, notably cocain and novocain, possess no antiseptic powers whatever, while others, notably apothesin and benzyl alcohol, exhibit distinct antiseptic effects.

3. These findings are deemed to be of interest not only in relation to healing of wounds, but also in respect to a direct effect on the flora of certain organs, notably the bladder and urethra.

II. A Study of the Antiseptic Action of Benzyl Alcohol and Local Anesthetics Against the Gonococcus

From the standpoint of the clinician, it would be distinctly advantageous if the solutions used as local anesthetics in the urethra and bladder were at the same time distinctly antiseptic. The possession of definite antiseptic value in the anesthetic would do away with the necessity of a preliminary sterilization of the solutions. The anesthetic solution for local use in urology must be nontoxic, the dangers attending the use of cocain in the urethra being well-known. There is need of an antiseptic anesthetic which can be incorporated into a lubricant for use on instruments used in the urethra.

In the acute inflammations of the urethra—a great majority of which are due to the gonococcus—the injection of an antiseptic even in weak solutions is often so painful that the injection method of treatment has to be abandoned till after the acute symptoms have subsided. Without entering into a discussion as to the advantages and disadvantages of the injection method of treatment, it would seem that the use of an antiseptic, which is also an anesthetic, and nontoxic, would permit a relief of the pain on urination and an earlier treatment of these cases.

In this study, no attempt has been made to determine the minimum time or minimum concentration of the drugs killing the gonococcus in a given period of time, but we have attempted only to ascertain the antiseptic and germicidal action of these compounds in strengths or concentration usually used in clinical work.

TECHNIC EMPLOYED

In making this study, the method described by Davis and Swartz[2] was employed. Fresh cultures of the gonococcus, twenty-four hours old, were used. The primary cultures were made from a case of acute gonorrhea in the male, the organisms being grown on veal infusion hydrocele agar at reduced oxygen tension after the method introduced by Swartz[3] and used exclusively in the laboratories of the Brady Urological Institute. The organisms were examined by Gram stain and sugar fermentation reaction to determine their identity. The organisms were washed from the agar slants by the use of warm normal salt solution—using about 5 c.c. of saline solution for each slant—and the resulting gonococcus suspension kept on the water bath at 37.5 C. Different strains of organisms were used in tests made on different occasions, and controls were always made both at the beginning and end of each experiment to be sure of the viability of the organism and insure accuracy of results.

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[2] Davis, David M., and Swartz, Ernest O.: The Testing of Germicidal Substances Against the Gonococcus. Forthcoming publication.

Drug dilutions were made in warm physiologic salt solution and a mixture of drug solution and gonococcus suspension in varying proportions gave our final drug dilutions. Care was taken to use enough of the gonococcus suspension to give plenty of gonococcus in the tube for transference to the media for incubation.

After the organisms had been immersed in varying strengths of drug solutions for three minutes, on a water bath, they were centrifuged in a high power centrifuge for one minute, the supernatant fluid poured off, the precipitated organisms washed with normal salt solution by stirring, and again centrifuged. The precipitated organisms were transferred to gonococcus media slants, the oxygen tension reduced, and the tubes quickly incubated. The washing with salt solution was to remove the antiseptic and prevent its transference to and subsequent inhibition of growth on the media. The work was so arranged that five minutes was the maximum time of contact of drug and organism.

Cultures were incubated for twenty-four hours at 37.5 C. and the results noted. If a single colony was present, it was counted as a failure of the drug to kill *all* the organisms. Cultures were incubated for twenty-four hours longer and results again noted. If colonies appeared after the second incubation period, it was considered that the late appearance was due to an antiseptic or inhibitory action of the drug on the organism.

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[3] Swartz, Ernest O.: Culture Methods for the Gonococcus: A Report of Experimental Studies, J. Urology **4:** 4, 325.

The slants on which there was no growth at the end of forty-eight hours were incubated for five days more to rule out the possibility of prolonged inhibitory action of the drug. At the end of this time, slants on which no growth of gonococcus had appeared were stroked with fresh viable gonococcus on a platinum loop and again incubated for forty-eight hours. The object of this procedure was to determine whether the organisms had been killed by the drug during the five minute contact period, or whether their growth had been inhibited by the small amounts of drug carried over to the medium on the loop, acting over the longer incubation period.

DISCUSSION

The method employed gives the germicidal value and does not give the inhibitory or antiseptic value, which is usually greater than the germicidal value. The test is rigid and favors the organism rather than the drug. If only one colony appears, it is counted as a failure of the drug at the given dilution to kill *all* the organisms.

The time chosen for contact between drug and organism—five minutes—probably does not represent the maximum time for antiseptic action in the urethra, and it is generally accepted that the germicidal value of many drugs is directly proportionate to the time of contact. While test tube experiments can give nothing but a rough evaluation of antiseptic values, yet from these tests it seems reasonable that our figures giving these values are low rather than high.

In these experiments, the temperature of the bacterial emulsions, physiologic salt solutions and solutions of drugs was maintained constant at 37.5 C. The number of organisms used in each test was approximately the same. The age of the cultures was the same, and the culture media used both for primary culture and for transplants was of the same composition, reaction and consistency. The importance of maintaining these factors constant was emphasized by Chick and Martin [4] who showed that the resistance of a given bacterial culture to antiseptics is modified by its previous history and that an increased incubation temperature (within the limits of growth) increased the resistance of the organisms.

TABLE 4

	Benzyl Alcohol, Strength of Solutions						Apothesin, Strength of Solutions					
	C	3 %	2 %	1.5 %	1 %	C	C	2 %	1.5 %	1 %	0.5 %	C
24-hour reading	+	0	0	+*	+	+	+	0	0	0	0	+
48-hour reading	+	0	0	+	+	+	+	0	0	0	+†	+
7-day reading	..	..	..	..	..	..	..	..	..	..	..	..
Tubes inoculated												
24-hour reading	..	+	+	..	..	..	..	+	+	+	..	..
48-hour reading	..	+	+	..	..	..	..	+	+	+	..	..

C, control tube; +, growth of gonococcus; 0, no growth of gonococcus.

* Three colonies. † Five colonies.

Solutions of the following drugs were studied: Alpha eucain, beta eucain, holocain, alypin, apothesin and benzyl alcohol.

Cocain and novocain solutions were found to be without any antiseptic action against the colon and staphylococcus and were not tested against the gonococcus.

A study of tables, included in this paper, shows definite antiseptic or germicidal action of all the drugs in certain concentrations, except beta eucain.

Alpha eucain, an isomere of beta eucain, inhibits the growth of gonococcus for the first twenty-four hours, but does not kill them. Only a few colonies survived in the 1 per cent. solutions, while the 0.5

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[4] Chick and Martin: Investigation of the Laws of Disinfection, Jour. of Hygiene **8**: 92-158, 654-697, 1908.

per cent. solution permitted a very luxuriant growth. Beta eucain solutions neither killed nor inhibited growth of gonococcus in this period of time in the dilutions used, which are concentrations usually used in clinical work.

Alypin killed the gonococcus in 5 per cent. solutions, but permitted a growth in the 2.5 per cent. solutions. There was no inhibitory action noted in case of this drug. Alypin solutions have a reaction of p_H 4.5 on the hydrogen ion scale. The work of Davis and Swartz[5] showed that plain acid solutions, not of themselves germicidal, will kill the gonococcus in twenty minutes if of a hydrogen ion concentration more acid than p_H 4.5, but that the gonococcus could survive immersion in warm solutions of acids of a hydrogen ion concentration of p_H 4.5 for twenty minutes. Hence, the germicidal action of alypin is not due to the acidity of its solutions, but to some other factor.

TABLE 5

	Alpha Eucain Strength of Solutions				Beta Eucain Strength of Solutions			
	C	1 %	0.5 %	C	C	1 %	0.5 %	C
24-hour reading	+	0	0	+	+	+	+	+
48-hour reading	+	+	+	+	+	+	+	+
7-day reading	..	..	..	..	..	..	..	..
Tubes inoculated								
24-hour reading	..	..	..	..	..	..	..	..
48-hour reading	..	..	..	..	..	..	..	..

C, control tube; +, growth of gonococcus; 0, no growth of gonococcus.

Holocain hydrochlorate solutions have an acidity of p_H 1.5—approximately that of tenth normal acid, and its germicidal action is probably due to its acidity, rather than to any specific action of the drug. Solutions of tenth normal acid kill the gonococcus in a few minutes.

Apothesin killed the gonococcus in five minutes in strengths of 1, 1.5 and 2 per cent. or over. In 0.5

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[5] Davis, David M., and Swartz, Ernest O.: The Prophylaxis of Gonorrhea. Forthcoming publication.

per cent. solutions, a few colonies survived. These did not appear for forty-eight hours, showing an inhibitory or antiseptic action even in 0.5 per cent. solutions.

Benzyl alcohol invariably killed the gonococcus in five minutes in strengths of 3 and 2 per cent. In one experiment, a few colonies survived immersion in 1.5 per cent. solution for five minutes. One per cent. solution did not kill the gonococcus in five minutes.

Benzyl alcohol is of neutral reaction, having a hydrogen ion concentration of p_H 7.0 and the germicidal action is not dependent on the acidity of the solution.

TABLE 6

	Holocain Strength of Solutions				Alypin Strength of Solutions			
	C	2 %	1 %	C	C	5 %	2.5 %	C
24-hour reading................	+	0	0	+	+	0	+	+
48-hour reading................	+	0	0	+	+	0	+	+
7-day reading..................	..	..	..	..	..	..	..	..
Tubes inoculated on 7th day								
24-hour reading............	..	+	+	..	..	+	..	..
48-hour reading............	..	+	+	..	..	+	..	..

C, control tube; +, growth of gonococcus; 0, no growth of gonococcus.

The antiseptic action of benzyl alcohol together with its lack of intoxicity, suggested its use as a gonococcocide, in acute gonorrhea. Work along this line is being carried on at present, and will be reported later.

After an incubation of seven days, all the tubes which showed no growth were inoculated with fresh gonococcus on platinum loop. All these tubes gave a growth of gonococcus, showing that there was no inhibition due to transference of drug to media on the platinum loop.

CONCLUSIONS

1. The germicidal value of certain concentrations of a number of local anesthetics against the gonococcus was determined. The solutions were of the same strength as those commonly used in clinical work.

2. Solutions of beta eucain have no antiseptic or germicidal action against the gonococcus. Alpha eucain solutions are antiseptic but not germicidal for the gonococcus.

3. Holocain, alypin, apothesin and benzyl alcohol are both antiseptic and germicidal for the gonococcus.

SOME OBSERVATIONS ON THE PHARMACOLOGY OF A DIGITALIS BODY *

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(From The Journal A. M. A., Aug. 14, 1920, p. 460)

The investigation with which this paper deals was started with a view to preparing a digitalis principle in a form suitable for intravenous administration following numerous inquiries concerning such a preparation. There seems, however, to be a more imperative need of one which is absorbed from the gastro-intestinal tract with greater uniformity than are the tinctures or other preparations made from digitalis obtained from a variety of sources and including wild grown and cultivated American, English and German leaves, partly from the first year's growth, partly from the second, and dried in a variety of ways.

The need of such a readily absorbable preparation has been called to my attention by the complaints of clinicians who were unable to secure the typical effects of digitalis even with large doses of the tincture in use in the hospitals with which they are associated. Several of these tinctures were submitted for examination, and in every case the biologic test showed that they were of a fair degree of activity.

Certain of the digitalis principles are readily absorbable from the gastro-intestinal tract of man, as well as that of animals, while others are absorbed much less readily, and it seems probable that the failures just mentioned arose from the fact that the preparations contained relatively large proportions of the less readily absorbable active principles.

A preliminary report[1] of a method of separating the more readily absorbable principles from those

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[1] Hatcher, R. A.: J. Am. Pharm. A. **8**: 913, 1919.

which are less readily absorbable was made about a year ago to the American Pharmaceutical Association. Since then modifications of that method have been employed in the effort to separate the more absorbable fraction in pure form and to estimate the amount in the leaf or tincture quantitatively. The substance obtained by the several modifications of the method is essentially the same, though it differs somewhat in purity. The simplest process for obtaining it consists in placing the wetted ground digitalis on a filter paper resting in water and supported by a wire frame, with a layer of chloroform beneath the water. The active principles of the leaf pass into the water by circulatory solution, and the chloroform takes up the more absorbable fraction, leaving the less absorbable in the water. The chloroform is then evaporated spontaneously, no heat or chemicals being used at any stage of the operation.

There is so much uncertainty regarding the chemistry of digitalis that any attempt at a comprehensive discussion of the subject would be out of place here; but certain salient points of resemblance between this chloroform-soluble substance and digitoxin, and certain dissimilarities may be pointed out. It is now generally accepted that digitalis contains digitoxin, digitalin, and one or two other active principles. Digitoxin is the only one of these that is soluble in chloroform, while all are readily soluble in alcohol, and none except the ill-defined digitalein is easily soluble in water. Nevertheless, water extracts all of the active principles of digitalis almost as readily as alcohol, a circumstance that affords evidence that these principles exist in the leaf in combination, and not in the free state as we know them.

The chloroform-soluble substance resembles digitoxin to a certain degree, both chemically and pharmacologically, and it seems probable that it contains combined digitoxin, though not in its original combination, because when once it is separated the substance is much less soluble in water than before;

and furthermore, when it is separated there remains another substance which is no longer soluble in water, and which is evidently thrown out of solution by the removal of the chloroform-soluble fraction.

The chloroform-soluble substance is resinous in character, brittle when cold, but softening with heat; it has a fragrant odor and an intensely bitter taste; it is soluble in chloroform and in alcohol in all proportions, and when water is added to a not too concentrated alcoholic solution the mixture remains clear or slightly fluorescent, though the substance is only slightly soluble in water; it is insoluble in petroleum benzin and in anhydrous ether, but ether dissolves a part of some specimens, leaving a part which is nearly indistinguishable in appearance and in pharmacologic action from the more soluble portion. If a fragment is dissolved in glacial acetid acid, a trace of ferric chlorid added and the solution allowed to flow over an equal volume of sulphuric acid contained in a test tube, without mixing, a dark zone appears at the line of contact, and the acetic acid gradually assumes a green, and then a blue, color, gradually fading to a dirty appearance, the reaction being identical with that given by digitoxin except that in addition the chloroform-soluble substance gives a ruby red color in the upper part of the sulphuric acid.

The behavior of digitoxin following its subcutaneous injection in the rat is peculiar, in that the animal, which excretes certain other digitalis bodies (ouabain and amorphous strophanthin) with extraordinary rapidity, shows a peculiar train of symptoms in which clonic convulsions are prominent, lasting for a period of about a week with just fatal doses. The symptoms following the subcutaneous injection of a just fatal dose of the chloroform-soluble substance are indistinguishable from those following similarly active doses of digitoxin. The rabbit eliminates a nearly fatal dose of digitoxin in a period of two or three hours in marked contrast to the cat and rat. It is significant that the chloroform-soluble substance is also eliminated by the rabbit at almost exactly the same rate as digitoxin. Such striking similarities of behavior in these two animals can hardly be a mere coincidence. The accompanying table gives certain points of resemblance between this chloroform-soluble substance and digitoxin.

One who has never worked with digitalis or other drug of similar complexity can have no adequate comprehension of the extraordinary variety of compounds which are obtained with slight variations in the method of extraction, while the changes in solubility of the active principles in different menstruums, resulting from slight differences in the impurities present, are confusing at times.

In marked contrast to the ease with which these combinations are broken down or altered is the stability of the most active of the principles which resists long continued boiling in weakly acid or neutral solution, and which I have obtained from a specimen of the fluid extract which had stood for nearly forty years. The active substance is associated with a saponin-like material, the greater part of which is separated easily but the last traces of which are removed only with some difficulty. It is difficult to remove traces of chlorophyl with which it is sometimes contaminated.

It was earnestly hoped that a method might be perfected for the quantitative determination of the chloroform-soluble fraction in digitalis leaves or the tincture in order that the dosage might be determined with reference to the content of this readily absorbable fraction, but the effort has not been successful. It appears to be merely a question of overcoming certain difficulties, and it is hoped that a satisfactory method of doing this may be devised.

The chloroform-soluble, or more absorbable, fraction and the aqueous residue, containing the less absorbable digitalis principles, are capable of exerting the typical digitalis action as shown on the frog, in which they induce ventricular systolic standstill, preceded by slowing, irregularity and dissociation of auricles and ventricle, and also by an electrocardiogram (for which I am indebted to Dr. R. Edwin Morris of the University of Minnesota) which shows a typical lengthening of the P-R interval with small doses and complete dissociation of the auricles and ventricles with large doses, together with a gradual flattening and inversion of the T wave in the cat. It also causes emesis through stimulation of the vomiting center in the cat and dog.

COMPARISON OF THE CHLOROFORM-SOLUBLE FRACTION OF DIGITALIS WITH DIGITOXIN

	Digitoxin	Chloroform-Soluble Substance
	White crystalline powder	Greenish; resinous
	Melts at 240 C.	Melts gradually at about 100 C.
	Odorless	Fragrant
	Intensely bitter	Intensely bitter
	Solubilities	
Alcohol	Easily soluble	Very soluble
Chloroform	Very soluble	Very soluble
Ether	Insoluble	Insoluble
Petroleum benzin	Insoluble	Insoluble
Water	Insoluble	Nearly insoluble
Color reaction	Positive	Positive (with ruby red)
Action in rat	Peculiar, hyperexcitability	Indistinguishable from that of digitoxin
Elimination, rabbit	Extraordinarily rapid	Extraordinarily rapid
	Fatal dose per kilogram (approximate)	
Cat, vein	0.35 to 0.5 mg.	1.0 mg.
Rabbit, vein	1.0 mg.	2.5 mg.
Frog, lymph sac	8.5 mg.	1.0 mg.

The relative absorbability of the two fractions (the chloroform-soluble, and the chloroform-insoluble) was studied in the following manner: They were assayed biologically by the cat method, which determines the amount required per kilogram of weight when injected intravenously to kill the animal, such an amount being called for convenience a cat unit. The fractions were then administered to cats through a stomach tube in doses varying from one to four cat-units per kilogram of weight, and the symptoms induced were observed. The results were not uniform, but the chloroform-soluble fraction proved to be much the more readily absorbable. The chloroform-soluble fraction is relatively less actively emetic than the residue, apparently, the intravenous injection of about 30 per cent. of the average fatal dose of the residue causing emesis in cats, while about 40 per cent. of the average fatal dose of the chloroform-soluble fraction is required; but the oral doses of as little as one cat unit per kilogram of weight of this fraction usually caused emesis in a short time, whereas oral doses of three and four cat units per kilogram of the aqueous residue sometimes failed to produce any perceptible symptoms. On the other hand, the absorption of the aqueous residue was more irregular than that of the chloroform-soluble fraction, and of two animals that received one, and two and a half, cat units per kilogram, respectively, of the same specimen of the residue the one receiving the smaller dose was nauseated and depressed, while the other showed no perceptible effects. The persistence of action in each case was determined on the following day by the differential test with ouabain (difference between the average fatal dose for a normal animal and that actually required for these cats); and while a single test is not conclusive, the results supported the evidence of absorption just mentioned; the cat which received the larger dose of the aqueous residue required much less of the ouabain than the one which had had the smaller dose. It may be remarked parenthetically, that we have found that the absorbability of several of the digitalis bodies from the gastro-intestinal tract of the cat closely parallels that observed in man.

An effort was made to estimate the relative amounts of the two fractions in a tincture and in a fluidextract which had been sent for examination because of their failure to induce the typical digitalis actions in man when used clinically in large amounts. Though it is impossible to separate these fractions quantitatively, it is at least significant that both of these preparations yielded very low percentages of the more absorbable fraction, and there is little reason to doubt that this fact explains why they proved ineffective in man.

We are especially concerned with the uniformity of action of the tinctures which we use by any given method af administration, and not with the presence in them of a variety of substances, including acids, volatile oils, saponins, resins, chlorophyl, sugar and coloring matter not to mention different active principles of unknown and varying degrees of activity; but since different tinctures vary in their absorbability, and therefore in their activity when administered orally, even though they may be of uniform activity when tested biologically, there is need of a preparation of as nearly uniform absorbability and activity as can be obtained. I wish to lay special stress on the fact that if one could always use the same tincture of digitalis, one would encounter little difficulty with its use after he had learned the dose, and he would probably never have an occasion to use such a preparation as the one now under consideration.

It is with this fact in mind that I present this paper dealing with a substance which I believe to be the most active principle of digitalis in a form more closely resembling that in which it exists in the leaf than any that has been prepared hitherto.

A digitalis body[2] designed for intramuscular or intravenous injection must meet the following requirements: It must be soluble in water, or at least in a weakly alcoholic menstruum; it must be of uniform activity; it must exert a minimum of undesired side actions; it must not deteriorate rapidly, and it must not irritate the veins or tissues into which it is injected

It is commonly assumed that the highest degree of purity is essential for a preparation which is injected intravenously; but while such a degree of purity is highly desirable, it is not an essential, provided the impurities present are not injurious. Tinctures of digitalis from different sources vary widely in their composition; nevertheless they have been injected intravenously with apparently satisfactory results. If clinicians have felt impelled to use the tincture in this way despite its disadvantages, it is obvious that any true digitalis body which possesses the essentials already mentioned must be preferable to the tincture.

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[2] While the terms "digitalis body" and "principle" are used in this paper, it is to be understood distinctly that the substance under consideration is not an alkaloid or glucosid in pure form. The substance probably consists of digitoxin mainly, and it is probably in combination much as it exists in the leaf, although this is only a surmise.

The chloroform-soluble substance meets the first of these requirements in that it is readily soluble in alcohol and is then readily miscible with water, the solution thus formed being fairly stable; the uniformity of activity is assured by the biologic assay; it is about as ectively emetic as tincture of digitalis, certainly not materially more so; so far as can be determined it does not deteriorate more rapidly than tincture of digitalis; the veins of cats were examined some days after toxic doses had been injected into them and found to be free from evidences of irritation, and numerous doses were injected into one of the ear veins of a rabbit during a period of several weeks. No evidences of pain could be elicited following the intramuscular injection into cats, but the following experiment on man is worthy of notice: An eighth of a milligram of the active substance, equal to an eighth of a cat unit, in 2,000 parts of physiologic sodium chlorid solution containing about 20 per cent. of alcohol, was injected into the deltoid (of the author). After about ten minutes there was a slight burning pain lasting for about twenty minutes. When this had become imperceptible during rest, a sudden violent movement of the arm was made with the purpose of determining whether pain could be elicited. There was a sharp burning pain lasting a minute or two, but this could not be elicited on repeating the movement. There was a slight sense of soreness during the following twenty-four hours when the arm was used, but this was not sufficient to attract the notice. The deltoid is more sensitive than the gluteus, but the dose was much smaller than that required for the full therapeutic effects; hence it is impossible to state whether the pain of the intramuscular injection of a therapeutic dose into the gluteal or lumbar region would be too painful or not.

From the foregoing it seems reasonable to conclude that the weakly alcoholic solution is suitable for intravenous, and possibly for intramuscular, injection in man, but that its ready absorbability following the oral administration renders other modes of administration superfluous in nearly all cases.

SUMMARY

Water exhausts ground digitalis leaves, and from the infusion thus prepared chloroform removes a principle which resembles digitoxin somewhat, both chemically and pharmacologically, and which is more readily absorbable from the gastro-intestinal tract of the cat than is the residue remaining in the infusion.

The chloroform-soluble substance is soluble in alcohol, and the alcoholic solution is miscible with water without precipitation, the weakly alcoholic solution undergoing little change in a period of about a year during which it has been under observation.

It is a true digitalis body, in that it exerts the typical cardiac action of the latter. It is not more actively emetic than is digitalis in proportion to its therapeutic effects.

It is believed that it is suitable for intravenous, and probably for intramuscular, injection, in the relatively few cases in which such methods of administration may be necessary.

It is hoped that a way be found to estimate the amount of this readily absorbable fraction in digitalis so that the powdered drug, the tincture or the infusion may be used instead of this chloroform-soluble fraction, which is naturally more expensive than the official preparations.

Further pharmacologic studies of the substance are in progress in the hope that the preparation may be made official, should the clinical studies show that it possesses any advantages over the official tincture.

THE ABSORPTION OF A DIGITALIS BODY

PRELIMINARY OBSERVATIONS *

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(From the Journal A. M. A., Aug. 14, 1920, p. 463)

The greatest diversity of opinion exists relative to the rate and completeness of absorption of digitalis from the human alimentary canal, and very few accurate observations have been made in this important field. In a study of the dosage of digitalis [1] published five years ago, it was shown that the tincture and the infusion, and commercial crystalline digitoxin, were generally absorbed both rapidly and fairly uniformly from the gastro-intestinal tract of man when adequate doses were administered. The precise rate of absorption was not determined, but the fact was brought out that the absorption of a single dose of digitalis was apparently complete within about six hours after its ingestion. It was also proved that full digitalization could be brought about by oral administration within from ten to twenty hours from the beginning of treatment. These observations have been confirmed by White and Morris,[2] and by Robinson.[3]

Robinson showed that the initial effects of digitalis appear in from two to five hours after the oral administration of a single large dose of the tincture, and in an unpublished report on the rapidity of absorption of digitalis from the digestive tract of man, H. E. B. Pardee concluded from his observations that the absorption of a single dose of the tincture is practically complete in about six hours, while a marked degree of absorption is almost always demonstrable in from two to four hours.

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* Read before the Section on Pharmacology and Therapeutics at the Seventy-First Annual Session of the American Medical Association, New Orleans, April, 1920.

[1] Eggleston, Cary: Digitalis Dosage, Arch. Int. Med. **16**: 1 (July) 1915.

[2] White, S. M., and Morris, R. E.: The Eggleston Method of Administering Digitalis, Arch. Int. Med. **21**: 740 (June) 1918.

[3] Robinson, G. C.: The Rapidity and Persistence of the Action of Digitalis on Hearts Showing Auricular Fibrillation, Am. J. M. Sc. **159**: 121 (Jan.) 1920.

These and other observations indicate that, as a general rule, digitalis is rapidly and fairly well absorbed from the human alimentary tract; and the fact that an average oral dose could be established shows that absorption generally takes place with a considerable degree of uniformity. This is borne out by an analysis of the full therapeutic doses recorded in sixty-six cases representing all forms of heart disease, collected from the papers of Pardee [4] and myself, in which only two, or 3 per cent., of the patients showed excessive variation from the average, and in both of these the administration was continued over a considerable period of time. One of these patients required nearly two and one half, the other three, times the average dose.

Admitting the occasional occurrence of a patient who proves unusually tolerant to digitalis through one or another unknown cause, there remains the much more important occurrence of specimens of digitalis which are for some inherent reason poorly absorbed, irrespective of the patient. Thus, Dr. B. S. Oppenheimer [5] records three patients who consumed enormous amounts of a given sample of digitalis without evidences of either therapeutic or toxic action. One of his patients took 540 minims of the tincture and 21 ounces of the infusion (same digitalis) in twenty-three days, equivalent to 134 c.c. of the tincture. A second took 3,090 minims (193 c.c.) of the tincture in thirty-eight days, and the third took 3,735 minims (233 c.c.) of the tincture (or its equivalent in infusion) in forty-three days. These amounts are from five to nine times the maximum average dose required for a sample that is absorbed as well as the average. Even when allowances are made for the long periods over which administration was continued, it is evident that the particular lot of digitalis employed was very poorly absorbed. Similar observations are reported by Wedd,[6] who records five patients who took of a given sample from four to six times the average total dose with no toxic symptoms, and with little or no therapeutic effect. Other instances have come to our attention, but we have insufficient data to warrant their inclusion; nor would anything be added by rehearsing them, since the foregoing are sufficient to indicate how poorly some samples of digitalis are absorbed.

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[4] Pardee, H. E. B.: Notes on Digitalis Medication, J. A. M. A. **73**: 1822 (Dec. 13) 1919.

[5] Oppenheimer, B. S.: Personal communication to the author.

The observations reported by Hatcher,[7] in which he shows that digitalis can readily be separated into two distinct fractions by extraction of its aqueous solution with chloroform, and his observations on the marked differences in the absorbability of the two fractions from the alimentary canal of the cat, opened the way for the clinical study of the absorption of digitalis from a new angle. This study, though begun some months ago, has not progressed as rapidly as was hoped, partly owing to the crippling of our service by the influenza epidemic. I regret, therefore, that I can at present make only a preliminary report, which, however, shows results in close harmony with those obtained by my colleague, Dr. Hatcher, on animals.

In order first to compare the absorption of each of the two fractions with the results previously obtained with various samples of tincture of digitalis made from high grade leaves, the fractions were supplied to me in the form of artificial tinctures in a menstruum of 60 per cent. alcohol. All the samples were prepared so that 1 c.c. represented one cat unit in activity. These were administered to patients and the whole procedure was made to parallel as closely as possible the original plan followed in the determination of the average total human dose of the tinctures. In this way the results could be compared directly with those secured from the use of the official tinctures.

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[6] Wedd, A. M.: Observations on the Clinical Pharmacology of Digitalis, Bull. Johns Hopkins Hosp. **30**: 131 (May) 1919.

[7] Hatcher, R. A.: Some Observations on the Pharmacology of a Digitalis Body, J. A. M. A. **75**: 460, 1920.

Twenty-three complete courses of administration were carried out in twenty-one patients, using four different samples of the chloroform-soluble, and one of the chloroform-insoluble, fractions. Each of the chloroform-soluble fractions, 8-3-19, 9-23-19 and 2-3-20, was obtained from a different lot of digitalis leaves, while the fraction 3-18-20 was made by mixing the chloroform-soluble extracts obtained from a large number of different samples of digitalis. The chloroform-insoluble fraction 9-23-19 was obtained from the same leaf as the chloroform-soluble fraction of the same number.

AVERAGE FULL THERAPEUTIC DOSES AND PERCENTAGE VARIATIONS FROM THE AVERAGES, FOR EACH FRACTION, FOR ALL CHLOROFORM-SOLUBLE FRACTIONS COMBINED, FOR TINCTURES OF DIGITALIS, AND FOR CRYSTALLINE DIGITOXIN

Preparation	Full Therapeutic Dose, Average	Number of Cases	Per Cent. Above	Per Cent. Below
Chloroform-soluble fractions:				
8-3-19	0.153	3	11	20
9-23-19	0.161	6	26	28
2-3-20	0.157	5	20	18
3-18-20	0.182	1	..	..
All chloroform-soluble fractions	0.159	15	28	26
Chloroform-insoluble fractions:				
9-23-19	0.640	4	19	30
Tinctures	0.148	25	97	66
Digitoxin*	0.066	15	66	43

* One very tolerant patient is included in this group; if omitted, the average for the fourteen remaining cases is 0.063, with maximum variations of 35 per cent. above and 40 per cent. below.

The determination of the average total therapeutic dose of each of the chloroform-soluble extracts was the first problem attacked, since its solution would provide the necessary data for the investigation of other problems. The full therapeutic dose of one sample of the chloroform-insoluble fraction was also determined approximately. The results of this phase of the investigation are set forth in the accompanying table, in which the corresponding figures for the tincture of digitalis (seven different specimens) and for crystalline digitoxin are included for comparison.

Reference to the table shows that the average full therapeutic dose of each of the first three chloroform-soluble extracts studied is approximately the same. The average total dose, calculated from fifteen instances in which one or the other of the four different samples of extract was used, is but slightly higher than the corresponding average for tinctures, as determined from twenty-five cases. The activity of the chloroform-soluble fraction for man, therefore, is not materally different from that of the average high grade tincture of digitalis, when the comparison is made on the basis of the biologic activity of each as determined by the cat method of assay.

The chloroform-soluble extract contains an average of about 1 mg. of solid per cat unit, while the amount of solid per cat unit of the official tincture of digitalis varies widely—between 28 and 44 mg. in three samples tested. These figures give some idea of the degree of purification of the extract. This is further shown by the fact that commercial crystalline digitoxin has an activity of from 0.35 to 0.4 mg. per cat unit, or only about three times that of the chloroform-soluble extract. Dr. Hatcher has shown that the extract gives the typical color reaction for digitoxin, and that it presents many close resemblances to digitoxin pharmacologically. In spite of these close similarities, he has shown that the chloroform-soluble extract of digitalis is not the same as crystalline digitoxin, but that it is probably a somewhat altered combination of digitoxin. It is of interest in this connection, therefore, to compare its behavior in man with that of crystalline digitoxin. The table shows that for man the activity of digitoxin is about two and one-half times that of the purified extract, when the comparison is made in terms of the biologic activity of each as determined by the cat method. When, however, the activity for man is compared in terms of weight, it is found that crystalline digitoxin is about seven times as active as the purified extract. These comparisons make it certain that the chloroform-soluble extract is not the same substance as crystalline digitoxin, and confirm the results of the laboratory investigations previously cited.

Turning now to the question of the absorption of the chloroform-soluble extract, it is seen from the table that the range of variation of dose from the average is much smaller for each of the three fractions investigated, and for all combined, than it is for representative good tinctures of digitalis. Thus, for the chloroform-soluble fraction the greatest divergence from the average dose amounts to only 28 per cent. of the average; that is, one patient of the fifteen developed full therapeutic effects only after he had received 28 per cent. more than the average dose required. This contrasts strikingly with the variation noted among a variety of tinctures obtained from different sources, which rises to 97 per cent. above, and falls to 66 per cent. below, the average. Further, while no variation greater than 28 per cent. of the average dose occurred in a series of fifteen patients receiving the chloroform-soluble fractions, six patients receiving the various tinctures of digitalis showed variations greater than 40 per cent. of the average dose, in a series of twenty-five.

These facts show that the chloroform-soluble extract of digitalis is absorbed from the human alimentary tract much more uniformly than are high grade tinctures of digitalis obtained from different sources, and it should be recalled that some samples of the official tincture obtained from reliable sources are so poorly absorbed that amounts from four to nine times the average full therapeutic dose fail to produce any demonstrable effects, or at most produce only slight and insufficient effects. The fact that the chloroform-soluble extract is absorbed from the human digestive tract with much greater uniformity than is the tincture of digitalis is alone sufficient to render it a valuable advance in the pharmacy of digitalis, even if it should prove to have no other advantages over the cruder preparations. Although at present no definite statements can be made on the basis of clinical investigation, it seems probable that the chloroform-soluble extract will prove to have other advantages, especially on account of its relative freedom from impurities and its ready miscibility with water, so that it should be available for intravenous injection.[8]

Before leaving the subject of the irregularity of the absorbability of commercial tinctures of digitalis which are derived from different sources, I want to call attention to the fact that the biologic activity of the tincture is not necessarily related to its absorbability from the human alimentary tract. This is well illustrated by the fact that the tinctures which Oppenheimer and Wedd found to be so poorly absorbed were both of approximately average biologic activity, and both were considerably more active than some of the tinctures which I found to be absorbed fairly well and relatively uniformly. It seems certain that this fact will necessitate a radical change in the method of assay of digitalis for human use, and Dr. Hatcher is now approaching the problem of determining both the activity and the absorbability of digitalis as a means of reaching a more accurate estimate of its therapeutic value.

The precise rate of absorption of the chloroform-soluble extract has not yet been determined for man, but the evidence so far obtained indicates that it is absorbed at least as rapidly as the most readily absorbed tinctures, and I can cite one case which was examined at least every hour and in which the maximal therapeutic action had developed within two hours and a half after a single dose amounting to about 0.112 cat unit per pound (15 c.c. total). The persistence of action of this extract has also not yet been determined for man, but again the evidence so far obtained indicates that it is equal to the best digitalis in this respect.

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[8] The chloroform-soluble extract has since been administered intravenously to one patient with satisfactory results and without untoward effects.

The chloroform-soluble extract has been supplied to several others for the study of its behavior in man, and we have reports from two who have used it in several cases. Dr. B. S. Oppenheimer of New York states that "the purified tincture of digitalis has proved uniformly satisfactory." Dr. R. W. Scott of Cleveland has administered it in eight carefully controlled cases and reports that "(1) digitalis action occurred in from one to two hours after 10 c.c. [single dose] was given by mouth. The certainty with which one could predict a definite improvement in the circulation in from eight to ten hours was indeed striking. . . . (3) Thus far the therapeutic effects have been fairly uniform as indicated by the level to which the heart beat was slowed, with resulting circulatory improvement."

I want to mention the results of the administration of the chloroform-insoluble extract to five patients, two of whom also received the chloroform-soluble extract from the same leaf on another occasion. The average, full therapeutic dose of the chloroform-insoluble fraction is about four times that of the chloroform-soluble, in terms of biologic activity, indicating that in man as well as in the cat it is absorbed very poorly following oral administration. Other studies on its behavior in man have not been made as yet, owing to the obvious lack of value of the preparation.

SUMMARY

1. The fairly rapid and complete absorption of digitalis from the human alimentary canal is shown for a number of different high grade tinctures of digitalis.

2. Contrasted with this is the very poor absorption of certain tinctures, of which from four to nine times the average therapeutic dose failed to produce therapeutic effects.

3. The marked uniformity of absorption of the chloroform-soluble extract of digitalis prepared from a variety of different leaves is shown and contrasts sharply with the variations noted in the cases of tinctures from a variety of sources.

4. The chloroform-soluble extract is shown to be absorbed at least as rapidly as the best tincture of digitalis, and its persistence of action is apparently of the same order as that of digitalis of the best grade.

5. The observations indicate that for oral administration the chloroform-soluble extract is not superior to a well absorbed tincture of digitalis, but it is far superior to tinctures which are derived from a variety of sources, the absorption of which shows very marked variations when the individual specimens are compared.

6. The chloroform-insoluble extract is very poorly absorbed from the human alimentary tract, as well as from that of the cat.

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ABSTRACT OF DISCUSSION

Dr. Henry A. Christian, Boston: Digitalis is a very good drug. Its use can be improved by investigation. We are getting better products to use in our practice, and consequently this sort of work is of very great importance. Although digitalis is not a perfect drug, it is not fair to blame the drug too often for getting poor results. Even a perfect drug will not give results unless it is used with brains. Digitalis is more often misused than almost any other drug. You can take almost any preparation of digitalis, use it with intelligence, and you can get results in almost any condition. The average doctor does not get the best results because he does not use the drug with intelligence. There is a lack of knowledge as to how to use the drug, and there is a lack of observation as to whether the drug is producing the result it should produce. It is the failure to know whether we are getting the desired results and to guide ourselves by that observation as to whether or not to increase the dosage, which largely is responsible for not getting desired results from digitalis. With these better preparations of digitalis and better methods of using them, poor results are going to be greatly reduced in number, but remember they are not going to disappear because no one can supply you with the intelligence that you may lack, or having it, fail to use.

Dr. Leonard G. Rowntree, Minneapolis: Within the last six or seven years we have obtained digitalis effects that we never saw before. I remember quite well when we first used Hatcher's method of standardization in Baltimore. We visited the hospital pharmacy, secured various preparations of digitalis leaves and assayed them by Hatcher's cat method. There was a variation in potency of 400 per cent. in the digitalis leaves which we found there. This emphasizes the aboslute necessity of using standardized preparations of digitalis in our clinical work.

I had a striking experience in relation to the introduction of standardized digitalis into clinical work. One day my intern called my attention to some digitalis effects. The charts showed a remarkable drop in the pulse rate. On investigation it developed that it was the new standardized preparation of digitalis, and these were the first cases in which it had been used. We can get infinitely better results therapeutically if, in all instances, we insist on having digitalis that has been standardized. The second great development in digitalis therapy came with the dosage introduced by Dr. Eggleston. Results with standardized preparations, but with ordinary doses, come at the end of forty-eight hours. With Dr. Eggleston's method the same results can be obtained in from eight to twelve hours. This is a tremendous advance, since many patients die because of the time required to elicit the effects of the drug. Dr. Eggleston's dosage unquestionably has saved a very large number of cardiac cases. The marked variation in absorbability is a matter of surprise to me. This may be due to the fact that we have been using exclusively the Minnesota preparation, and, in a large proportion of cases, the Eggleston dosage. In our work in Minneapolis we have never experienced difficulty from lack of absorption.

Dr. Cary Eggleston, New York: If one can always obtain a good preparation of digitalis there is no need for the refinements introduced in this chloroform-soluble extract. Dr. Rowntree is extremely fortunate in being able to work with a preparation of digitalis which is grown and cared for with the greatest of attention from the beginning until it comes into his hands as a finished product, so that he has a preparation which is of fairly uniform activity and uniformly well absorbed. The preparations generally used come from every conceivable source and are made with every conceivable degree of care and lack of care. So the products, while standardized, may or may not be readily absorbed. We have had several specimens submitted that were irregular in absorption and very poorly absorbed. The more we see of these the more we think there is something to be gained by the effort to reduce this irregularity in absorption, which we try to avoid by the use of the chloroform-soluble preparations described.

DIVISIONS OF THE SO-CALLED FLEXNER GROUP OF DYSENTERY BACILLI *

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(From the Journal of Experimental Medicine, Dec. 1, 1920, p. 651)

INTRODUCTION

There is considerable confusion in regard to the existence of subdivisions of the mannitol-fermenting group of dysentery bacilli. Numerous attempts have been made to establish subdivisions on the basis of their biological and serological characteristics.[1-8] My con-

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* From the Department of Pediatrics and the Department of Pathology of the Johns Hopkins University, and the Harriet Lane Home of the Johns Hopkins Hospital, Baltimore.

† For the technic employed and a description of the cases of dysentery in children, see Davison, W. C.: Bacillary Dysentery in Children, Bull. Johns Hopkins Hosp. **31**: 225, 1920.

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2. Andrewes, F. W.: Dysentery Bacilli: the Differentiation of the True Dysentery Bacilli from Allied Species, Lancet **1**: 560, 1918.

3. Levine, M.: Dysentery and Allied Bacilli, J. Infect. Dis. **27**: 31, 1920.

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6. Torrey, J. C.: A Comparative Study of Dysentery and Dysentery-Like Organisms. Part I, Bacilli Producing a Typical Reaction in Litmus Milk, J. Exp. Med. **7**: 365, 1905; Part II, Dysentery-Like Organisms Which Produce an Atypical Reaction in Litmus Milk and Which Should Probably be Included Within the group of Pseudodysentery Bacilli, 385.

7. Sonne, C.: Ueber die Bakteriologie der Giftarmen Dysenteriebacillen (Paradysenteriebacillen), Centr. Bakt., 1te Abt., Orig. **75**: 408, 1915. Hehewerth, F. H.: Ueber Dysenterie-Bacillen und ihre Einteilung in Grüppen, Centr. Bakt. 1te Abt., Orig. **68**: 3, 1916. Thjøtta, T.: On the Bacteriology of Dysentery in Norway, J. Bact. **4**: 355, 1919. Gettings, H. S., and Douglas, S. R.: An Investigation of the Flexner-Y Group of Dysentery Bacilli, Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 30, 1918.

8. Kruse, W.: Ueber die Ruhr als Volkskrankheit und ihren Erreger, Deutsch. med. Wchnschr. **26**: 637, 1900; Weitere Untersuchungen über die Ruhr und die Ruhrbazillen **27**: 370, 386, 1901; Die Blutserumtherapie bei der Dysenterie **29**: 49, 1903; Zur Geschichte der Ruhrforschung und über Variabilität der Bakterien, 201; Neue Unter-

clusions are derived from the ferementation and agglutination reactions of seventy-seven cultures isolated from cases of clinical dysentery in children, twelve cultures from cases of dysentery in the American Expeditionary Forces, and stock cultures of the five English types of Flexner bacilli.†

By the Flexner group I refer to Gram-negative nonmotile bacilli, isolated from the stools of cases of clinical dysentery and agglutinated by the patient's serum. They produce a small amount of indol, do not liquefy gelatin, fail to ferment lactose, and produce acid and no gas in dextrose and mannitol. These are the only constant cultural characteristics. It would seem advisable to apply one name, *Bacillus dysenteriae* Flexner, to this whole group. These mannitol-fermenting dysentery bacilli have been subdivided in two ways, first by fermentation tests with maltose, saccharose, dulcitol, and rhamnose, and secondly by agglutination tests with the serums of immunized rabbits.

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suchungen über die Ruhr **33**: 292, 338, 1907; Der jetzige Stand der Dysenteriefrage, Deutsch. Aerzte-Ztg. **1**: 25, 1902; Die Gesundheitsverhältnisse der Aerzte, Geistlichen und Oberlehrer im Vergleich mit denen anderer Berufe, Centr. allg. Gsndhtspflg. **19**: 229, 1900; Dienes, L.: Beobachtungen über das serologische Verhalten der giftarmen Dysenteriestämme, Z. Immunitätsforsch., Orig. **28**: 456, 1919. Douglas, S. R.; Colebrook, L., and Morgan, W. P.: Report upon a Series of Cases of Dysentery Received from the Mediterranean Expeditionary Forces, Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 6, 1917. Glynn, E.; Berridge, E. M.; Foley, V.; Price, M., and Robinson, A. L.: A Report upon 2,360 Enteritis "Convalescents" Received at Liverpool from Various Expeditionary Forces, Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 7, 1918. Park, W. H.: Personal communication. Park, W. H.; Dunham, E. K.; Collins, K. R., and Payne, W. A.: A Clinical and Bacteriological Study of a Number of Outbreaks of Disease Due to the Dysentery Bacillus of Shiga, Bull. Med. Sc. **2**: 187, 1902. Park, W. H.; Collins, K. R., and Goodwin, M. E.: The Dysentery Bacillus Group and the Varieties which Should be Included in It, J. Med. Research **11**: 553, 1904. Park, W. H., and Carey, H. W.: The Presence of the Shiga Variety of Dysentery Bacilli in an Extensive Epidemic of Dysentery, J. Med. Research **9**: 180, 1903. Park, W. H.: On the Interpretation of Reactions of Agglutination Among the Bacilli of Dysentery, Med. Rec. **63**: 358, 1903. Martini, E., and Lentz, O.: Ueber die Differenzirung der Ruhrbacillen mittels der Agglutination, Z. Hyg. u. Infectionskrankh. **41**: 540, 1902. A Study of the Serological Races of the Flexner Group of Dysentery Bacilli, Med. Research Com., Nat. Health Insurance, Special Rep. Series, No. 42, 1919. Winslow, C.-E. A.; Kligler, I. J., and Rothberg, W.: Studies on the Classification of the Colon-Typhoid Group of Bacteria with Special Reference to Their Fermentative Basis, J. Bact. **4**: 429, 1919.

DIVISIONS BASED ON CARBOHYDRATE FERMENTATION

In this country it has been customary to refer to the Flexner,[9] Harris, Hiss-Russell,[10] and Strong[11] divisions on the basis of differences in the fermentation of maltose and saccharose. In my series, by utilizing the differences of fermentation of dulcitol and rhamnose, three additional divisions were found in a few cases (Table 1). Other workers using still different carbohydrates have reported additional divisions.[6, 12-14] In fact, if fermentation is accepted as a criterion for differentiation, still further members will probably be added in the future as new carbohydrates are used for fermentation tests, and even more confusion will result.

It has been demonstrated[7, 15] that dysentery cultures may lose or gain ability to ferment maltose, saccharose, and also dulcitol[4] and that distinctions made on this basis are inconstant and unreliable. In the same stool I have isolated cultures that fermented maltose and others that failed to do so (Case 53, Table 2). In another dysentery specimen I have found organisms that differed in dulcitol fermentation (Case 8, Table 2). In other stools I have isolated dysentery bacilli that differed in rhamnose fermentation (Cases 13 and 79, Table 2).

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[9] Flexner, S.: On the Etiology of Tropical Dysentery, Philadelphia Med. J. **6**: 414, 1900; The Etiology of Tropical Dysentery, Centr. Bakt., 1te Abt. **28**: 625, 1900; A Comparative Study of Dysenteric Bacilli **30**: 449, 1901; A Comparative Study of Dysentery Bacilli, Brit. Med. J. **2**: 786, 1901; On the Etiology of Tropical Dysentery, Bull. Johns Hopkins Hosp. **11**: 231, 1900. Flexner, S., and Barker, L. F.: Report of a Special Commission Sent to the Philippines by the Johns Hopkins University to Investigate the Prevalent Diseases of the Islands, Johns Hopkins Univ. Circ. **19**: 13, 1900.

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TABLE 1.—TYPES OF FERMENTATION OF FLEXNER DYSENTERY BACILLI

Fermentation Type	Name	Motility	Fermentation of Test Substances									Per Cent. of Cases
			Indol	Gelatin	Lactose	Dextrose	Mannitol	Dulcitol	Rhamnose	Maltose	Saccharose	
1	Hiss-Russell...........	0	+	0	0	+	+	0	0	0	0	7
2	Flexner, English Y....	0	+	0	0	+	+	0	0	+6	0	41
3	Strong.................	0	+	0	0	+	+	0	0	0	+13	14
4	Harris, English V, X..	0	+	0	0	+	+	0	0	+	+15	24
5	English Z.............	0	+	0	0	+	+	0	+12	+4	+12	7
6	B. alkalescens.........	0	+	0	0	+	+	+5	0	+	0	3
7	....	0	+	0	0	+	+	0	+	0	+6	3

If late fermentation occurred, the day of its appearance is designated by the number placed after the + sign. Otherwise + designates acid (no gas) production on the first day and indol production on the seventh day; 0 denotes no fermentation (incubation continued 21 days).

Fermentation Type 7 was also isolated from a diarrhea case which was not clinically dysentery.

It is, of course, possible that these represent mixed infections, but this is impossible to prove, inasmuch as the fermentation differences in maltose, saccharose, dulcitol, and rhamnose, as will be pointed out later, do not parallel the serological findings.

DIVISIONS BASED ON AGGLUTINATION WITH MONOVALENT RABBIT SERUMS

Murray[1] in England has discarded the fermentation divisions of the Flexner group and has found that thirty-four cultures isolated from cases of dysentery in different parts of the world fell into five divisions on the basis of their agglutination reactions with

TABLE 2.—DISCREPANCIES BETWEEN FERMENTATION AND AGGLUTINATION DIVISIONS OF FLEXNER DYSENTERY BACILLI IN CHILDREN

Case No. (Culture No.)	Agglutination Type of Stool Culture					Summary	
	Type Sera					Fermentation Type	Agglutination Type
	V	W	X	Y	Z		
53*† (L156), 6*, (69, 70, 73)	0	+125	0	±200	0	1	W,Y
6* (72, 74)	±200	0	0	0	0	1	V
39* (L61), 40* (L56, L57, L58)	0	0	0	±200	0	2	Y
39* (L51), 40* (L59), 46* (L82), 53*† (L154), 80* (L11), 10* (161, 174, 175A)	0	0	0	0	0	2	0
46* (L83, L84), 52 (L166, L167), 54*† (L155), 54 (L159)	0	+250	+250	±500	0	2	W.X Y
80* (L9)	±125	±250	0	±300	0	2	V,W,Y
80* (L10, L12), 81 (L30, L32, L33), 8*† (107, 113, 114, 115, 116), 10* (156, 159, 162, 164, 186, 157)	0	±250	0	±250	0	2	W,Y
9 (153, 154)	+1,250	0	0	0	±500	2	V,Z
Y stock	+125	0	+250	+625	±100	2	V,X,Y,Z
58 (L174, L175)	0	0	0	0	0	3	0
77 (L186, L187)	0	0	±1,250	+200	±1.250	3	X,Y,Z
5 (67)	+500	0	±200	0	±500	3	V.X.Z
V stock	+1,250	0	0	+250	0	4	V,Y
X stock	0	0	+250	0	0	4	X
41* (L65)	0	0	0	0	0	4	0
41* (L66) 45* (L75, L77), 63* (L114)	0	0	+500	0	+250	4	X.Z
41* (L67)	0	0	0	0	±200	4	Z
41* (L68, L69), 13*† (262, 268)	±200	0	+200	0	+250	4	V.X.Z
45* (L76)	0	+100	+250	±200	0	4	W.X,Y
63* (L115, L116), 78 (L188)	0	0	+500	+200	+150	4	X,Y,Z
79*† (L2, L5), 13*† (261, 263, 266, 270, 271, 274)	±1,000	0	0	0	±250	4	V,Z
13*† (260)	+1,250	0	0	±200	±250	4	V,Y,Z
13*† (267), 79*† (L4)	+500	0	0	0	±500	5	V Z
Z stock	0	0	+625	0	+625	5	X,Z
8*† (108)	0	0	0	0	0	6	0
69 (L111, L112), 39 (152)	0	0	0	0	0	7	0

Cases marked with an asterisk (*) had more than one serological type and those marked with a dagger (†) had more than one fermentation type of Flexner dysentery bacillus in the stools. Culture numbers preceded by L, i. e., L156, were from cases in Baltimore, the others were from Birmingham, Ala. + and ± indicate complete and partial agglutination; the numbers following these signs refer to the dilutions (end-point) of the serum. Case 69 was a diarrhea which was not clinically dysentery.

monovalent rabbit serums. These he designates as the V, W, X, Y, and Z divisions of the Flexner group.

As Murray emphasizes, these divisions are not definite but probably indicate that one or more antigens predominate in a given strain. With the English cultures and serums, by means of Dreyer's[16, 17] technic with formolized cultures, I have found that although each serum usually agglutinates its own organism to higher titer than other types (Table 3), yet the V serum will also agglutinate the Y culture, the X serum will agglutinate Y and Z, the Y serum will agglutinate the V culture, the Z serum will agglutinate the Y culture, while the W serum, which is of low titer, appears to be specific. Murray states that absorption tests do not make these serological divisions more distinct. It may perhaps be found that agglutination with the serums of animals other than the rabbit may give more clear-cut results, as has been demonstrated[18] with sheep serum for streptococci.

With the cultures which I have isolated there is even more cross-agglutination (Tables 2 and 4). Some cultures that are agglutinated to end-titer by V serum react to quarter titer with Z. W, X, and Y serums frequently agglutinate the same cultures. With other cultures further combinations were found.

Among 62 cultures from children, of which many reacted with more than one serum (Table 2), there were 21 positive agglutination reactions with V serums, 28 with W, 21 with X, 38 with Y, and 28 with Z, while 15 cultures failed to agglutinate with any of these serums. It is probable that at least two of these fifteen inagglutinable cultures (Nos. 174 and 175, Tables 2 and 4) represented a type differing from Murray's five, for the patient's serum (Case 58, Table 4) had no agglutinins for the V, W, X, Y, and Z

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[16] Dreyer, G.: Widal's Reaction with Sterilized Cultures, J. Path. and Bact. **13:** 331, 1909; On Anvendelse af dræbt kultur til Widal-Reaktion, Hospitalstid. **14:** 532, 4. R., 1906; Proceedings of the British Medical Association, Section of Pathology, Discussion on Immunity, Brit. Med. J. **2:** 564, 1904.

[17] Davison, W. C.: The Diagnosis of Enteric Fever (Typhoid and Paratyphoid A and B) by Agglutination Tests, J. A. M. A. **66:** 1297, 1916; Report on Prophylactic Vaccination with B. Typhosus, B. Para typhosus A, and B. Paratyphosus B, J. Lab. and Clin. Med. **2:** 607, 1916-17.

[18] Dochez, A. R.; Avery, O. T., and Lancefield, R. C.: Studies on the Biology of Streptococcus. I. Antigenic Relationships Between Strains of Streptococcus Hemolyticus, J. Exp. Med. **30:** 179, 1919.

stock cultures. Further artificial cultivation might have rendered the others agglutinable, but the destruction of the laboratory and cultures by fire prevented it.

In other words, although a distinct division was not obtained, agglutination reactions with the five English type serums and these mannitol-fermenting dysentery bacilli lead to the assumption that different antigens exist and that there are more than five which must be recognized.

TABLE 3.—CROSS AGGLUTINATION OF THE FIVE ENGLISH TYPE SERA

		Agglutination Titer					
Serum	Culture	1:100	1:125	1:250	1:625	1:1,250	1:2,500
V	V	+	+	+	+	+	0
	W	0	..	..	..	..	..
	X	0	..	..	..	..	..
	Y	+	+	0	..	..	..
	Z	0	..	..	..	..	..
W	V	0	..	..	..	..	..
	W	+	+	+	0	..	..
	X	0	.	..	..	..	..
	Y	0	..	..	..	..	..
	Z	0	.	..	..	..	..
X	V	0	..	..	..	..	..
	W	0	..	..	..	..	..
	X	+	+	+	0	..	..
	Y	+	+	+	0	..	..
	Z	+	+	+	+	0	..
Y	V	+	+	+	0	..	..
	W	0	..	..	..	..	..
	X	0	..	..	..	..	..
	Y	+	+	+	+	0	..
	Z	0	..	..	..	..	..
Z	V	0	..	..	..	..	..
	W	0	..	..	..	..	..
	X	0	..	..	..	..	..
	Y	+	0	..	..	..	..
	Z	+	+	+	+	0	..

DISCREPANCIES BETWEEN FERMENTATIVE AND SEROLOGIC DIVISIONS

There is no correlation between the divisions by fermentation tests and the serological results. Organisms of the same fermentation reactions were agglutinated by different serums. As an example (Table 2),

TABLE 4.—CORRELATION OF PATIENT'S SERUM REACTIONS AND TYPES OF STOOL ORGANISMS

		Patient's Serum Reactions						Agglutination Type of Stool Culture					Summary		
		Type Cultures						Type Sera							
Case No.	Length of Time After Onset, Days	V	W	X	Y	Z	No. of Culture from Stool	V	W	X	Y	Z	Type of Patient's Serum Reaction	Agglutination Type of Stool Culture	Fermentation Type of Stool Culture
39	200	0	0	0	±50	0	61	0	0	0	±200	0	Y	Y	2
							51	0	0	0	0	0	..	0	2
40	205	±200	±200	0	±200	0	56, 57, 58	0	0	0	±200	0	V,W,Y	Y	2
							59	0	0	0	0	0	..	0	2
46	21	+250	+1,000	+100	+250	±20	83, 84	0	+100	+250	+500	0	V,W,X,Y,Z	W,X,Y	2
							82	0	0	0	0	0	..	0	2
54	14	+100	0		+100	0	159	0	+250	±200	±500	0	V,Y	W,X,Y	2
80	19	+ 20	±20	0	+200	±20	9	±125	±250	0	±300	0	V,W,Y,Z	V,W,Y	2
	38	+100	±50	0	±200	±20	10, 12	0	±250	0	±250	0	....	W,Y	2
							11	0	0	0	0	0	..	0	2
58	6	0	0	0	0	0	174, 175	0	0	0	0	0	0	0	3
77	26	± 50	0	0	± 50	0	186	0	0	±1,250	+200	±1,250	V,Y	X,Y,Z	3
	76	+ 20	0	0	0	0	187	.....	.....	.....	.....	.....	V		
41	184	0	±20	0	0	0	66	0	0	+500	0	+250	W	X Z	4
							68, 69	±200	0	+250	0	+500	....	V,X,Z	4
							67	0	0	0	0	±150	....	Z	4
							65	0	0	0	0	0	..	0	4
63	68	0	0	+ 20	0	0	115, 116	0	0	+500	+200	+150	X	X,Y,Z	4
							114	0	0	+500	0	±150	....	X,Z	4
78	13	± 20	±20	0	+ 20	0	188	0	0	+1,250	+500	+500	V,W,Y	X,Y,Z	4
79	17	+200	0	0	+100	+20	2, 5	±1,000	0	0	0	±250	V,Y,Z	V,Z	4
	21	+100	0	0	+100	+50	4	±1,000	0	0	0	±250	....	V,Z	5
69		0	0	0	0	0	111, 112	0	0	0	0	0	0	0	7

+ and ± indicate complete and partial agglutination; the numbers following these signs refer to the dilutions (end-point) of the serum. 0 indicates no agglutination with the patient's serum at 1:20, and with the diagnostic type serums at 1:150.

among the group that ferment maltose and saccharose, usually called the Flexner-Harris division (Fermentation Type 4), some cultures were agglutinated only by the Z serum, some by the X, Y, and Z, some by the V and Z, some by the X and Z, some by the W, X, and Y, and still others by the V, X, and Z serums, etc. Conversely, organisms agglutinated by the same serums may have different fermentation reactions. It was also noted that among organisms from the same stool with the same fermentation reactions, some were agglutinated by one or more of the serums while others were inagglutinable.

With the English stock cultures (Tables 1 and 3) the same lack of correlation exists; for instance, the Y and X cultures differ in saccharose fermentation, yet both are agglutinated by the X serum.

Levine [3] has laid emphasis on the fact that the Z culture is the only one to ferment rhamnose, yet it is agglutinated by the X as well as the Z serum. Two rhamnose fermenters isolated from dysentery cases were agglutinated by both V and Z serums. Three rhamnose fermenters, isolated from cases of diarrhea which were not clinical dysentery, were not agglutinated by Z or any of the other serums. Furthermore, the Z serum agglutinated many strains that did not ferment rhamnose. From one stool (Case 79, Table 4) one organism that fermented rhamnose and two that failed to ferment it were agglutinated by V and Z serums.

Dulcitol-fermenting dysentery bacilli from one case were agglutinated by the Y serum and those from another were inagglutinable. This division has been named *Bacillus alkalescens* by Andrewes [2] who regards it as nonpathogenic.

CORRELATION OF AGGLUTINATION REACTIONS OF THE PATIENT'S SERUM AND FERMENTATION AND SEROLOGICAL TYPES OF HIS STOOL ORGANISMS

A partial correspondence exists between the serological type of the organisms isolated from the patient's stool and the agglutination reactions of his serum.

In all except one case (Table 4), if the patient's serum agglutinated any of the five stock antigens, the organisms isolated from the stool reacted with one or more of the serums of the same types. In many cases, however, the stool organisms were not agglutinated by all the types for which the patient had agglutinins. As an example, one patient's serum (Case 40, Table 4) had agglutinins for Types V, W, and Y, while the dysentery bacillus isolated from his stool reacted with only the Type Y serum.*

The reverse of this was also true; namely, that the patient's serum would not have agglusinins for all the types which were represented by his stool organisms; for instance, the organisms isolated from a patient's stool (Case 63, Table 4) were agglutinated by the X, Y, and Z serums, while his serum had agglutinins for only the X antigen. These illustrations indicate an overlapping of the antigenic content of these strains.

In the only instance in which there was no correspondence (Case 41, Table 4), the serum of the patient agglutinated the W culture while the organisms from the stool reacted with the V, X, and Z serums.

In a few cases in which tests were repeated after various intervals, if agglutinins persisted, they were of the same types that had been present in previous tests.

CONCLUSIONS

From these data it is seen that ill defined divisions of the so called Flexner group exist. The divisions do not appear to be sufficiently distinct to warrant the use of separate names. To avoid confusion all mannitol-fermenting dysentery bacilli should be called *Bacillus dysenteriae* Flexner and the subdivision noted. There are two methods for this division, one by the fermentation of carbohydrates, the other by agglutination with monovalent rabbit serum. These do not coincide and one or the other and not both must be adopted. Inasmuch as Murray studied organisms from widely distributed sources, it would seem preferable to adopt his serologic classification and to add to it the types that fail to be agglutinated by his V, W, X, Y, and Z serums, as this method is simpler and more rapid. The results of the agglutination reactions of the patient's serum may be expressed in the same terms as the serological typing of the organism from his stool. Fermentation is less constant and gives rise to more divisions than there are carbohydrates.

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* It might be argued that had more colonies been studied in the stool culture, Types V and W might have been found; but this would not explain the instances in which the patient's serum did not have agglutinins for all the types found in his stool.

TABLE 5.—AGGLUTININ CONTENT OF POLYVALENT THERAPEUTIC SERUM

		Agglutination Titer							
Culture	Serum	1:100	1:125	1:250	1:625	1:1,250	1:2,500	1:6,250	1:12,500
Polyvalent Therapeutic Serum	V	+	+	+	+	+	+	0	
	W	+	+	+	+	+	+	+	0
	X	+	+	0					
	Y	+	+	+	+	+	+	+	0
	Z	+	+	+	+	+	0		

The serologic reactions of these type serums, as Murray points out, show cross-agglutination to a greater or less extent, but they indicate that there are five antigens, V, W, X, Y, and Z and probably others, one or more of which predominate in a given strain. Polyvalent diagnostic and therapeutic serums are practically worthless unless they include antibodies for the more common of these types.

The diagnostic importance of recognizing that there are five or more antigens in this group is seen from the fact that the serums of some pateints react with one, others with another, and that unless several antigens are used, some positive tests may be missed.

The therapeutic importance is emphasized by the fact that probably the best polyvalent therapeutic serum at present available has a very low titer for the X antigen, although that type was found in many of these cases (Table 5).

A STUDY OF MERCURY INJECTIONS BY MEANS OF THE ROENTGEN RAY *

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(From The Journal A. M. A., Dec. 4, 1920, p. 1559)

The intramuscular route of mercury administration has been popular for many years in the treatment of syphilis. If properly and carefully used, the method is cleanly and efficient, and does not lead to detection by outsiders. Nevertheless, the intramuscular method has its faults, the most important being uncertain, uneven and delayed absorption of the mercury, especially when insoluble preparations are being used. Consequently, insoluble injections have been responsible for most of the fatalities reported from the use of mercury in the treatment of syphilis. Thus, Wolffenstein,[1] in 1913, reviews all the cases of therapeutic mercury fatalities that could be found in the literature since 1883, some 108 in number. Of these deaths only one was the result of oral medication due in that case to five pills of 0.06 gm. each of mercury. There was one fatality from inhalation and there were nineteen from inunctions. Of the fatalities, the author reported that seven were from soluble injections and seventy-eight from insoluble, thirty-one of the latter being due to the use of gray oil, oleum cinereum.

Notwithstanding the importance of the subject, there are few data as to the course of the absorption

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* From the Departments of Dermatology and Syphilology of the Western Reserve University School of Medicine, and of the Lakeside and Cleveland City Hospitals; and from the Department of Pharmacology, Western Reserve University School of Medicine.

[1] Wolffenstein, W.: Berl. klin. Wchnschr. **50**: 1904 (Oct. 13) 1913.

of insoluble mercury injections; and the few available data are far from satisfactory. It therefore seemed desirable to make a thorough clinical study of the absorption of these injections. This succeeded by the use of roentgen-ray plates, which gave perfect pictures of the unabsorbed insoluble mercury preparations in the tissues. By taking these at proper intervals, the absorption can be ascertained as closely, as often and as long as desired.

We were not aware that any attempt along this line had been made in the past. Subsequently, however, we found that Fournier [2] reports that Oudin and Barthelemy made roentgenograms of gray oil injections. They found it to be disseminated in the muscles in long spindles made up of globules, the spindles being from 1 to 4 cm. in length. In forty-eight hours these spindles were broken and shorter, and on the fourth day scarcely any traces of the metal were left. We may say at once that this is not confirmed by our results. As we have not seen the original data, we shall not undertake to explain the discrepancy.

Our investigation was made with the usual clinical doses, using a sufficient number of patients to avoid experimental accidents and difficulties. Some patients would pass out of our control before their series was completed; some others did not give clear negatives because they were too fleshy, etc. There was also the difficulty of obtaining successive exposures from the same angle. However, it will be seen that the number of cases that remain is ample to justify conclusions, especially as the results are so nearly uniform.

The investigation included both insoluble and soluble injections, mercuric chlorid being generally in doses of about one-eighth grain and red mercuric iodid in doses of from one-sixth to one-third grain; the insoluble injections of calomel and mercuric salicylate in doses of from 1 to 2 grains; and 40 per cent. gray oil in doses of from 0.125 to 0.25 c.c.

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[2] Fournier, A.: Treatment of Syphilis, English translation, New York, Rebman Company, 1906, p. 143.

Muscular men were found the most suitable subjects; only a few women were used, as the adipose tissue in the buttocks gave imperfect pictures.

RESULTS WITH SOLUBLE MERCURY SALTS

Solutions of red mercuric iodid or mercuric chlorid do not in themselves throw a marked shadow. When mixed with albumin or serum a shadow is thrown when the mercury is precipitated, as with the mercuric chlorid; but not in the absence of precipitation, as with the red mercuric iodid. In other words, only insoluble mercury compounds throw a shadow. With intramuscular injection, neither the mercuric chlorid nor the red mercuric iodid throw a perceptible shadow undoubtedly because even the mercuric chlorid precipitate is rapidly redissolved by the tissues. In other words, roentgen-ray method is not suited to the study of the absorption of soluble injections.

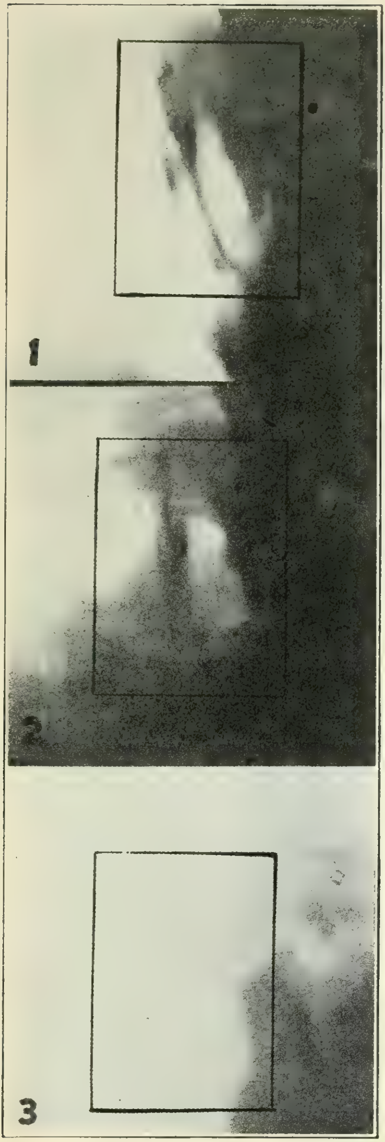
In six different patients we obtained negative results five times; and in one man a faint shadow showed in the arm following an injection of red mercury iodid. It went away, however, in twenty-four hours. In still another patient who had received the red mercuric iodid injections into the buttocks over a long period of time no shadow was revealed by the roentgen ray.

MERCURY OINTMENT INUNCTIONS

We made several roentgenograms of mercury inunctions on the skin, but they show nothing.

INSOLUBLE INJECTIONS

Insoluble injections gave very definite and quite unmistakable shadows. The mercuric salicylate injections show on roentgenograms as a long streaked blotch. With the calomel this blotch is even sharper. With the gray oil one is able to make out the separate metallic globules of varying sizes. After a time there is a tendency of the globules to spread out and even to obey the laws of gravity and settle down in the tissues somewhat.



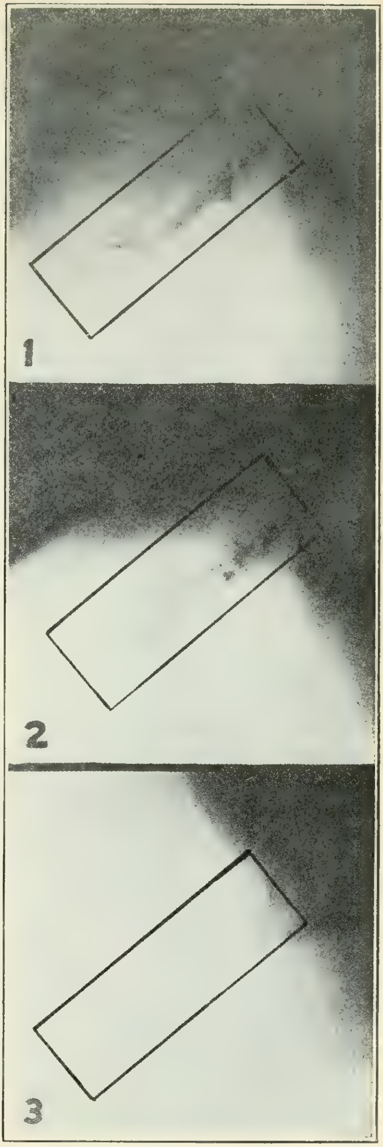
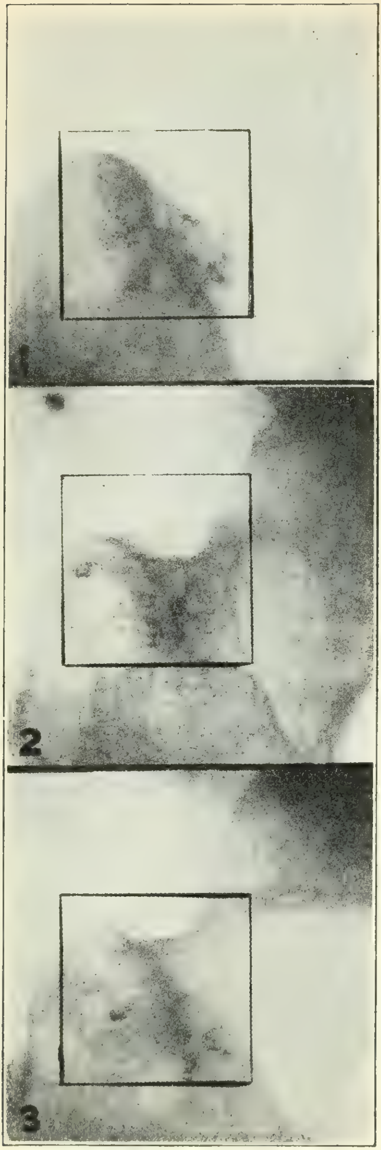


Fig. 1.—Mercuric salicylate, 2 grains: 1, fresh in lumbar muscles; 2, two days later; 3, seven days later; all absorbed.

Fig. 2.—Calomel, 2 grains: 1, fresh in buttock; 2, six days old; 3, eighteen days old; no shadow.



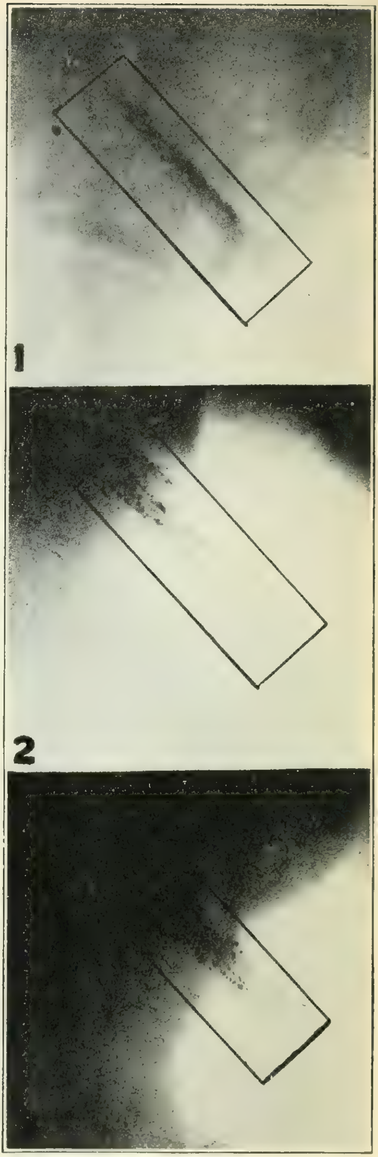


Fig. 3.– Calomel, 2 grains: 1, fresh in buttock; 2, ten days old; 3, nineteen days old; still unabsorbed.

Fig. 4. Gray oil (40 per cent. mercury), 0.25 c.c.: 1, fresh in buttock; 2, four days old and globules beginning to spread; 3, 125 days old; still unabsorbed.

MERCURIC SALICYLATE

In our first experiments, mercuric salicylate was used as a 10 per cent. suspension in liquid petrolatum. With most of them, however, we employed a 10 per cent. suspension in lanolin emulsion, as suggested by Nitardy,[3] which keeps the powder in a much more permanent suspension, and at the same time avoids the danger of paraffin tumors. To this was added camphor and creosote, which materially diminish the pain and soreness. The combination has now been used on a large number of patients without any ill effects, and appears to be a material improvement over the usual mixture.

FORMULA FOR PERMANENT AND ANESTHETIC MERCURIC SALICYLATE SUSPENSION

	Gm. or C.c.
Anhydrous lanolin	40
Distilled water	10
Sweet almond oil	150
Calomel or mercuric salicylate	29
Phenol or creosote	20
Camphor	40

Dose: 1 c.c. equals 0.09 gm. (1½ grains) of mercuric salicylate or calomel.

With twelve different patients receiving a dose of mercuric salicylate of from 1 grain to 1½ grains in the buttocks, we found that the roentgen-ray shadow had entirely disappeared in a space of 4, 4, 5, 4, 4, 10, 7, 3, 4 and 4 days. In two of the twelve patients the drug was almost gone in three days and one day, respectively, when the patient disappeared, making a mean of 4 days and the extremes of 4 and 10 days.

With twenty-two patients, the injections were made into the deep *lumbar muscles*. From these, the mercuric salicylate had disappeared in 11, 9, 10, 2, 7, 8, 17, 8, 4, 14, 4, 8, 4, 11, 7, 7, 11, 11, 4 and 13 days, and in two of the twenty-two it was practically gone in 12 days and 24 days, making a mean of 8½ days and the extremes of 2 to more than 24 days. The combined mean for the mercury salicylate is 7 days.

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[3] Nitardy: J. A. Pharm. A. **8**: 961, 1909.

The unmistakable faster absorption from the gluteal injections is worthy of note. It is perhaps due to the natural massage of pressure and position.

An average of 3½ satisfactory plates were made per patient.

CALOMEL

Twenty-five patients received injections of calomel, all into the buttocks. An average of 5.4 plates was made per patient. The shadow had disappeared in 18, 4, 8, 7, 9, 15, 19, 7, 6, 14, 14, 12, 19, 12, 17, 18, 15, 26, 19 and 11 days; and in three of the twenty-five patients at the end of 34, 37 and 39 days over half the calomel was still not absorbed, and in one patient it was not quite absorbed in 19 days. This gives the mean as 15 days and the extremes as 4 to over 39 days.

GRAY OIL (MERCURIAL OIL)

Twenty-seven patients received injections, all into the buttocks, with doses of from ⅛ to ⅙ c.c. of the 40 per cent. suspension. The observations were continued as long as the patients could be kept under observation, namely, for the following number of days: 54, 75, 44, 25, 28, 32, 37, 60, 47, 33, 25, 25, 71, 75, 42, 66, 16, 20, 74, 20, 38, 45, 60, 21, 61 and 125 days, a mean of 43 days and extremes of 16 and 125 days. Fourteen plates were used on one patient and 169 plates were used for the twenty-seven patients, an average of 6¼ plates per patient. In no instance with the gray oil did we find absorption completed at the end of this time, and in practically every case at least one half of the metallic globules were still present in the muscular tissue.[4]

These facts are in striking contrast with the deductions that have been made from chemical estimations on animals, by Schamberg, Kolmer and Raiziss.[5] However, they based their conclusions on a single experiment with each drug, and on doses that were about a hundred times as great as those used clinically; doses so large that even with the salicylate, 46 per cent. was unabsorbed after forty-five days.

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[4] Professor Todd, head of the Anatomy Department of Western Reserve University School of Medicine, informs us that they often find globules of metallic mercury in the buttocks of subjects in the dissecting room. This quite agrees with our findings.

[5] Schamberg, Kolmer and Raiziss: J. Cutan. Dis. **33**: 819-838, 1915

PRACTICAL APPLICATIONS

The results of these investigations make the intramuscular mercury injections appear in a rather different light.

The fact that the major part of the mercury of gray oil remains unabsorbed for four months, and presumably for indefinite periods, means that it is both ineffective and dangerous—ineffective, because the amount absorbed is ordinarily very small; dangerous, because the absorption may become temporarily improved, for instance, by accidental massage in sitting, etc. Repeated injections would increased the surface and thus the rate of absorption, and therefore both the efficiency and the dangers. The most important point is that with the present method of gray oil administration, the patient is loaded with a large store of mercury which does no good and may easily do harm. This is undoubtedly bad practice. If the use of gray oil is to be continued at all it should be given in very much smaller doses, so as to avoid the accumulation. It is doubtful whether a sufficiently even absorption or an adequate dosage could be secured by this means. In the meantime, however, we feel that the use of gray oil injections should be discontinued.

With calomel injections, the conditions are somewhat more favorable. However, the absorption of the ordinary doses requires on the average two weeks and sometimes five weeks, so that cumulative effects must occur when the injections are repeated with shorter intervals. This agrees with the clinical experience that calomel injections are one of the most efficient methods of mercury administration, but that there is also much danger of cumulative poisoning. Moreover, calomel is such a painful drug when used in injections that it is hardly necessary to bring it into the sphere of this paper, so far as recommending it for general use is concerned. It may perhaps have a restricted use in exceptional cases, but must be watched with the greatest care.

Mercuric salicylate, on the other hand, has stood the test very well. The absorption of the usual dose, from 1 to 2 grains, is completed on the average in four days when injected into the buttocks, and in nine days when injected into the lumbar muscles. It is therefore effective. The injections may be safely repeated with these intervals. However, the absorption is not uniform in all cases, so that even with the salicylate, the patient must be watched carefully for any toxic manifestations.

In treating a case of syphilis, we must remember that mercury is a very powerful drug and a dangerous poison, especially for the kidneys. Any patient who is receiving this drug should have the teeth and gums examined frequently by the physician, at least once a week. The physician should inquire as to symptoms of diarrhea and of griping pains in the bowels, and the urine should be examined weekly. On the appearance of the least trace of albumin in the urine the use of the insoluble mercury preparation should be stopped at once. When these precautions are taken we feel that mercuric salicylate injections are quite safe. We should recommend that they be employed according to the formula that we have mentioned.

CONCLUSIONS

1. The absorption of "insoluble" mercury preparations from intramuscular injections can be followed admirably by roentgen-ray shadows. The method is not applicable to soluble preparations.

2. An extensive study of clinical cases gave the following as the time when absorption is completed:

Mercuric salicylate: By gluteal muscles, mean, 4 days; extremes, 4 to 10 days. By lumbar muscles, mean 8½ days; extremes, 2 to beyond 24 days.

Calomel: Mean, 15 days; extremes, 4 to 39 days.

Gray oil: Unabsorbed during entire period of observation, a mean of 43 days; extremes of 16 to 125 days.

3. These findings indicate that gray oil injections are both inefficient and dangerous, and their use should be abandoned.

Calomel injections are also dangerous.

Mercuric salicylate injections, especially into the gluteal muscles, give a satisfactory absorption and present relatively little danger.

We have recently seen a case of poisoning from gray oil injections in which none of the drug had been received for four months. Roentgen-ray examination revealed large masses of metallic mercury globules.

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